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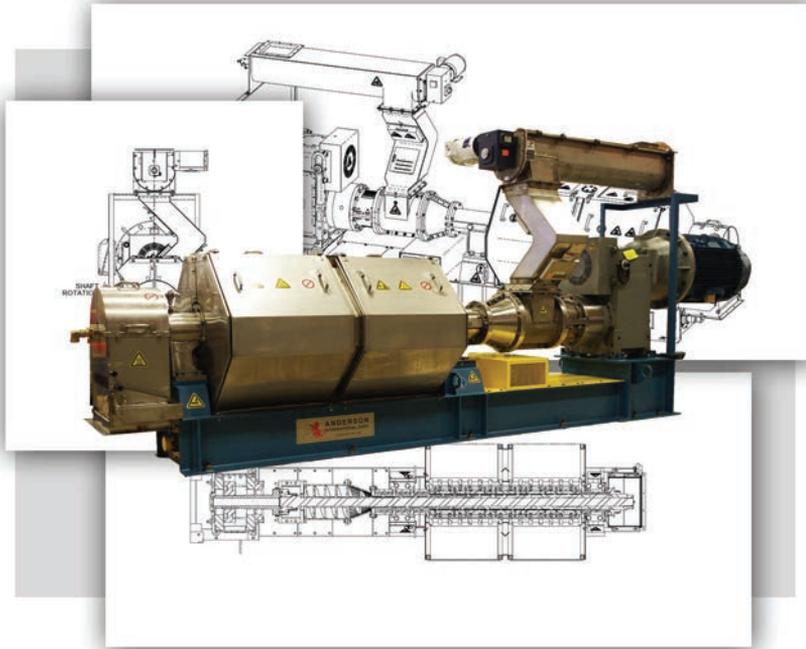


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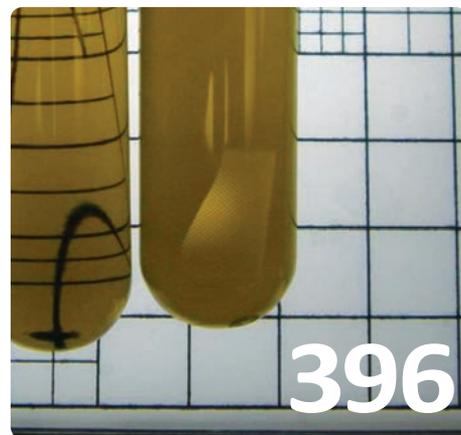


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# Big fat controversy: changing opinions about saturated fats

Laura Cassiday

- **Nutritionists have long vilified saturated fat for its propensity to raise LDL (“bad”) cholesterol levels in the blood.**
- **Although initial epidemiological studies associated saturated fat intake with heart disease risk, subsequent studies have failed to confirm the link.**
- **Saturated fat raises HDL (“good”) cholesterol levels, perhaps ameliorating its effects on LDL cholesterol.**
- **An unintended consequence of a low-fat diet may be increased carbohydrate intake, which could actually raise heart disease risk compared with a higher-fat diet.**

In the early hours of September 24, 1955, US President Dwight D. Eisenhower suffered a massive heart attack. The popular president and war hero was visiting in-laws in Denver, Colorado, where he had enjoyed 27 holes of golf before retiring early that evening with what he thought was indigestion. Although Eisenhower recovered and went on to win a second term in office, his sudden incapacitation heightened public awareness of the growing epidemic of cardiovascular disease. Once a rare ailment, by the 1950s heart disease had become the leading cause of death in the United States. What diet, lifestyle, or other factors were responsible for this dramatic change? People were looking for a scapegoat, and nutritional scientists were soon to provide one.

Researchers were already beginning to implicate dietary fats, particularly saturated fats, in cardiovascular disease. The logic went like this: Saturated fats such as those found in butter,

meat, cheese, and eggs raised serum cholesterol in laboratory animals and humans. Because cholesterol is a major component of atherosclerotic plaques, and early studies had linked high serum cholesterol levels to heart disease, then saturated fat must cause heart disease.

In 1970, well-known nutrition researcher Ancel Keys, who developed the US Army K-rations during World War II, published his famous Seven Countries Study (Keys, A., ed., *Circulation* 41(4 Suppl.):1–200, 1970). Keys compared the health and diet of 12,700 middle-aged men in Italy, Greece, Yugoslavia, Finland, the Netherlands, Japan, and the United States. His conclusion: Populations that ate large amounts of saturated fats in meat and dairy had more deaths from heart disease than those that ate mostly grains, fish, nuts, and vegetables.

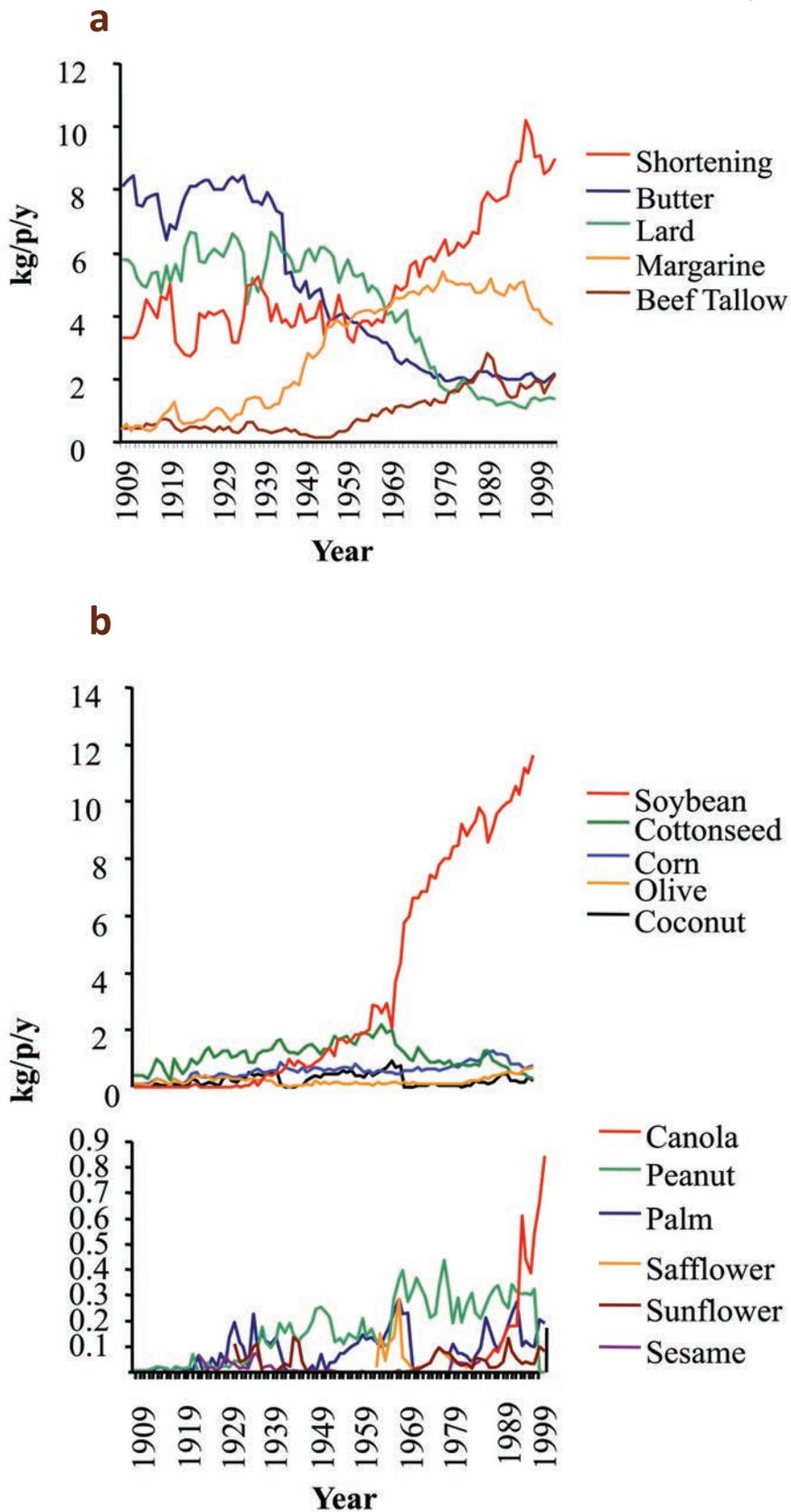
When the US Department of Agriculture (USDA) and the US Department of Health and Human Services jointly released the first *Dietary Guidelines for Americans* in 1980, saturated fat played a leading villainous role. The *Guidelines*, which are updated every five years by an expert committee and form the basis of US nutritional policy, advised citizens to “avoid too much fat, saturated fat, and cholesterol.” The United Kingdom issued similar dietary guidelines in 1984. Both guidelines recommended reducing overall fat consumption to 30% of total calories, and saturated fat to no more than 10% of calories—values that have remained essentially unchanged in subsequent iterations.

Yet recent evidence, as well as reevaluation of older studies, has questioned whether dietary fat is really as bad as the experts have been saying for the past three decades. A paper published in the January 29, 2015, edition of the *BMJ's Open Heart* examined the data on fat and cardiovascular disease available to US and UK regulatory committees at the time the 1980 and 1984 guidelines were issued (Harcombe, Z., et al., <http://dx.doi.org/10.1136/openhrt-2014-000196>, 2015). The analysis revealed that the six randomized controlled trials available back then did not provide sufficient evidence that cutting total fat or saturated fat intake reduces deaths from heart disease. The authors conclude that the “dietary advice not merely needs review; it should not have been introduced.”

Now, 35 years since the first guidelines were issued, a continued lack of consistent evidence showing that



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**FIG. 1** Trends in the apparent consumption of *a.* major fat categories between 1909-C and 1999, unadjusted for changes in total energy consumption; *b.* vegetable and seed oils between 1909-C and 1999, unadjusted for changes in total energy consumption. Reprinted with permission from Blasbalg, T. L., et al., "Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century." *Am. J. Clin. Nutr.* 93:950–962, 2011. DOI: 10.3945/ajcn.110.006643. © US Government.

dietary saturated fats cause heart disease, as well as an improved understanding of how fats affect different types of cholesterol particles in the body, has cast doubt on the government's recommendations. Some experts say it is time to increase or even nix the limit on saturated fat found in the *Dietary Guidelines for Americans*. Doing so would have far-reaching consequences, from changing school lunch programs to readjusting the priorities of food manufacturers. Although the Scientific Report of the 2015 Dietary Guidelines Advisory Committee recommends maintaining the status quo with regard to saturated fat, the official guidelines will not be released until the third quarter of 2015. Meanwhile, some experts argue that the current recommendations on saturated fat are not only ineffective at reducing rates of cardiovascular disease, obesity, and type 2 diabetes, they may actually be doing more harm than good.

## Changing diets

Before 1910, people in the United States used butter and animal fats almost exclusively for cooking and baking. These were rich in saturated fatty acids, which are defined chemically as fat molecules with no double bonds between carbon atoms of the hydrocarbon chain. Saturated fats are solids at room temperature. In contrast, most vegetable oils such as corn, soybean, and canola/rapeseed oils are liquids at room temperature and contain primarily unsaturated fats, either monounsaturated (one double bond in the hydrocarbon chain) or polyunsaturated (multiple double bonds). In 1910, cooking with vegetable oils was virtually unheard of—oils were instead used to make soaps, candles, lubricants, and other nonedible products.

But then a dramatic change occurred. As the process of hulling and pressing seeds and beans was mechanized, vegetable oils became cheaper than raising and slaughtering animals for butter or animal fat. Aggressive marketing by vegetable oil companies claimed that vegetable oils were a more healthful, easier-to-digest, and more sanitary alternative to animal fats. In 1911, Proctor & Gamble applied for a US patent for the process of hydrogenating vegetable oil; in other words, adding hydrogen molecules to remove some of the double bonds in unsaturated fatty acids. This process enabled the production of solid vegetable oils such as Crisco shortening and margarine, increasing the shelf lives of oils and paving the way for their use in baking and frying.

From 1909–1999, consumption of soybean oil in the United States increased by more than 1,000-fold per person and margarine consumption increased 12-fold, whereas consumption of butter and lard decreased by about four-fold each (Blasbalg, T. L., *et al.*, <http://dx.doi.org/10.3945/ajcn.110.006643>, 2011). These changes in consumption are depicted in Fig. 1.

Yet at the same time these supposedly “heart-healthy” changes were taking place, heart disease was on the rise. In the past decade, deaths from heart disease in the United States have dropped (Mozaffarian, D., *et al.*, <http://dx.doi.org/10.1161/CIR.000000000000157>, 2015), mainly due to reduced smoking and improved emergency care, but heart disease remains the No. 1 killer of people worldwide (World Health Organization, Fact Sheet No. 317, 2015).

Another major dietary change that has taken place in the past 50 years is the substitution of fats in the diet with carbohydrates such as pasta, grains, sugar, fruit, and starchy vegetables. According to Nina Teicholz, author of *The New York Times* bestselling book *The Big Fat Surprise: Why Butter, Meat & Cheese Belong in a Healthy Diet*, in 1960 approximately equal numbers of calories in the American diet came from fats and carbohydrates (40% each). Then, the low-fat diet craze hit the nation. People began avoiding foods such as full-fat dairy, eggs, and red meat and substituted low-fat or fat-free foods, many of which had added sugar to make them more palatable. Now, carbohydrates comprise about 50% of total calories in the US diet, while total fats are down to about 30%. Meanwhile, saturated fat consumption has dropped to about 11% of total calories (Wright, J.D., and Wang, C.-Y., NCHS Data Brief, No. 49, 2010).

Ironically, these values are right in line with the US government’s recommendations, yet obesity, heart disease, and diabetes continue to be problems. “The experts like to claim that Americans are fat and unhealthy because they don’t follow the guidelines—it’s their own fault,” says Teicholz. “But if you look at the broad data it’s very clear that, in terms of macronutrients, we have been following the guidelines.”

## Fat facts

The reason dietary fats garnered such a bad reputation in the 1950s is that a high fat intake, particularly saturated fat, raises the level of total cholesterol in the blood, which is a risk factor for heart disease. But it wasn’t until the 1980s that researchers began to appreciate that all forms of cholesterol are not created equal.

Cholesterol and other fats are transported in the bloodstream by different lipoprotein complexes. Low-density lipoproteins (LDL), or “bad cholesterol,” can contribute to plaques in the arteries, increasing the risk for cardiovascular disease. However, high-density lipoproteins (HDL), or “good cholesterol,” have the opposite effect: They transport cholesterol away from artery walls, reducing the risk of heart disease.

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- Berneis, K. K., and Krauss, R. M. (2002) "Metabolic origins and clinical significance of LDL heterogeneity." *J. Lipid Res.*, 43:1363–1379. <http://dx.doi.org/10.1194/jlr.R200004-JLR200>.
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The propensity of saturated fat in the diet to raise LDL cholesterol is what nutrition researchers have found so worrisome. In contrast, mono- and polyunsaturated fats tend to lower LDL cholesterol, which is why the *Dietary Guidelines* recommend replacing saturated fats in the diet with unsaturated fats. However, saturated fats also raise HDL cholesterol more than any other type of fat, possibly mitigating the harmful effects of LDL cholesterol. Trans fats, the intended replacement for saturated fats, raise LDL cholesterol even more than saturated fats, while lowering HDL cholesterol levels.

Total cholesterol levels in the blood do not always correlate well with a person's risk for heart disease risk because the measurement includes both LDL and HDL cholesterol. A more sensitive and specific predictor is the ratio of total cholesterol to HDL cholesterol (total:HDL) (Kinosian, B., et al., *J. Invest. Med.* 43:443–450, 1995). Mono- and polyunsaturated fats lower total:HDL cholesterol, suggesting that they reduce the risk of heart disease. In contrast, trans fats increase the ratio, presumably increasing the risk of heart disease. However, because of their effects on both types of cholesterol particles, saturated fats neither raise nor lower total:HDL cholesterol (Mensink, R. P., et al., *Am. J. Clin. Nutr.* 77:1146–1155, 2003), suggesting little or no effect on cardiovascular disease risk (Fig. 2, page 349).

In the 1990s, Ronald Krauss, director of atherosclerosis research at Children's Hospital Oakland Research Institute in Oakland, California, USA, and a practicing physician, discovered that the situation is even more complex than "good" and "bad" cholesterol. Krauss developed a technique to separate LDL cholesterol into different types of particles: large, buoyant particles and small, dense particles. As it turns out, the small, dense LDL particles are more strongly associated with cardiovascular disease risk than the large, buoyant particles (reviewed in Berneis, K. K., and Krauss, R. M., <http://dx.doi.org/10.1194/jlr.R200004-JLR200>, 2002). The small LDL particles are more easily oxidized and more likely to trigger inflammation and plaque formation, leading to atherosclerosis.

Krauss has studied the effects of diet on these two LDL subpopulations. "What we found is that the small, dense form of LDL is raised by carbohydrates, and the larger form is raised by saturated fat," he says. "And so we started to wonder if the dietary effects of saturated fat on LDL cholesterol could be misleading in terms of heart disease risk." Krauss notes that because the larger particles contain more cholesterol, they contribute more to the measurement of LDL cholesterol in the blood than the smaller particles. Therefore, total LDL cholesterol measurements, commonly used by doctors to gauge heart disease risk, could fail to identify the patients at highest risk.

Although methods for measuring specific types of LDL are now commercially available, the tests aren't widely utilized, says Krauss. "The testing is inexpensive, but it's not well known, and there's a lot of controversy regarding its clinical use," says Krauss. "But for people who are concerned about their heart disease risk, have other risk factors, or are considering treatments, it provides a much finer tool than the blunt

## Saturated and trans fat alternatives

That dietary guidelines in many countries continue to vilify saturated fats, along with more recent warnings about trans fats, has put many food manufacturers between a rock and a hard place. “Partially hydrogenated vegetable oils were meant to replace saturated fats,” says Nina Teicholz, author of *The New York Times* best-selling book *The Big Fat Surprise: Why Butter, Meat & Cheese Belong in a Healthy Diet*. “Now, because of the trans fat scare, the food industry can’t use partially hydrogenated vegetable oils, which the FDA is on the verge of banning anyway, and they can’t use saturated fats because people are so afraid of them.”

Solid fats are particularly needed in the bakery industry. “In bakery products, fat plays several roles beyond just flavor and succulence,” says Charles Speirs, baking science and technology manager at Campden BRI, a membership-based food and beverage research facility with headquarters in Gloucestershire, UK. “For example, in cakes you need saturated fats to help sustain the bubble structure that you get during rising and baking.” Speirs says that liquid vegetable oils do not have the same functional properties and therefore cannot replace saturated fats in products such as cakes, cookies, and pastries.

In his research at Campden BRI, Speirs has explored the use of next-generation emulsions to reduce saturated fat in bakery products. Ordinary water-in-oil emulsions don’t have the same properties as hard fats such as butter or lard. So Speirs and his colleagues developed techniques to fill water-in-oil emulsions

with an alginate gel derived from seaweed. “By a clever bit of chemistry, we can make an alginate gel in the aqueous phase, within a continuous oil phase, that gives you the body of a hard fat,” he says.

Speirs has had to adjust baking conditions to compensate for the higher moisture content of products containing the emulsion. However, he says that the mouthfeel of the bakery products is remarkably similar to versions containing higher amounts of saturated fat. “There is a lightening of the color, but certainly with the testing we’ve done, people don’t mind that,” he says.

Other food companies are turning to tropical oils such as palm oil to replace trans fats. Palm oil contains about 50% unsaturated and 50% saturated fats. According to Gerald McNeill, vice president of research and development at IOI Loders Croklaan, an edible oil supplier with US headquarters in Channahon, Illinois, palm oil is making a huge comeback after being decimated by anti-saturated-fat media campaigns in the 1970s. When McNeill first started working with palm oil, he thoroughly researched the literature on saturated fat. “If saturated fat was really that unhealthy and artery-clogging, I didn’t want to be involved with that,” he says. “I read all the literature, and I decided there was no evidence whatsoever that saturated fat has any effect on heart disease, good or bad.”

By heating palm oil to different temperatures and then cooling it and collecting the crystals that form, IOI Loders Croklaan has been able to separate palm oil into about a dozen fractions containing fatty acids with different properties, ranging from liquids to buttery textures to waxy solids. In addition, inter-esterification can be used to swap positions of fatty acids on the glycerol backbone, which confers additional functional properties to palm oil fractions.

McNeill and his coworkers can precisely match the physical properties of various partially hydrogenated oils used in baking and frying by blending the palm oil fractions in different proportions. “So far we’ve been able to match every partially hydrogenated vegetable oil that anybody has come to us to make a match for,” says McNeill.

Thus, many food manufacturers have come full circle, from saturated fat to trans fat to saturated fat again, albeit in a different form. “The solution for trans fat is saturated fat, which is laughable because trans fat was invented to replace saturated fat,” says McNeill.

LDL cholesterol measurement, which can really miss the boat in terms of LDL particle subtypes.”

Not everyone is convinced that particle size matters, however. “I don’t think we can say that any LDL is good,” says Penny Kris-Etherton, distinguished professor of nutrition at The Pennsylvania State University. “Some people are saying the large particles are less harmful, but the American College of Cardiology and the American Heart Association are saying just look at total LDL. All of it’s bad, and you want to get it down.” Kris-Etherton notes that the American Heart Association’s dietary recommendations for lowering

cholesterol include reducing saturated fat to only 5–6% of total calories, even lower than the 10% recommended by the USDA.

### The problem with epidemiology

When the first *Dietary Guidelines for Americans* were released in 1980, few clinical trials had been conducted on saturated fat and heart disease risk, and those that had

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## Dietary fat and cancer

The evolution in scientific thinking about dietary fat and cancer has followed a similar narrative to that of dietary fat and heart disease.

The diet–cancer connection was a corollary to the diet–heart hypothesis pushed by Ancel Keys and others from the early 1950s forward. In fact, a report in 1982 by the National Research Council (NRC) suggested the evidence supporting the association of dietary fat with cancer was so irrefutable that the report likened those researchers who remained skeptical with “certain interested parties [who] formerly argued that the association between lung cancer and smoking was not causal.”

To date, the most extensive literature review on cancer prevention is the continuing evaluation undertaken by the World Cancer Research Fund International (WCRF; [www.wcrf.org](http://www.wcrf.org)) and the American Institute for Cancer Research (AICR; [www.aicr.org](http://www.aicr.org)). The groups’ first report was published in 1997.

“At the time of the 1997 WCRF/AICR review, it was recognized that associations between dietary fat and risk of breast cancer seen in case-control studies had not been confirmed in prospective studies with substantial statistical power,” wrote a team of epidemiologists from the University of Oxford and Harvard University in *Public Health Nutrition* (<http://dx.doi.org/10.1079/PHN2003588>, 2004). “Similar differences in results have now been observed for fat intake in relation to incidence of colon and lung cancers.”

The most recent WCRF/AICR report on *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective* appeared in 2007 and reflects the evolution in thinking. The groups commissioned nine systematic literature review teams, each with 22 panelists, to synthesize the literature on nutrition, physical activity, and cancer. The panelists studied 7,000 articles, reviews, and meta-analyses in all languages. Team findings went to an international panel that synthesized information for many different cancers to come up with the report’s main recommendations.

The panel found that there is “only limited evidence suggesting that diets relatively high in fats and oils (in total, or any type) are in themselves a cause of any cancer.” As the report notes, this finding runs counter to previous reports. Overall, the panel decided that the evidence on fats and oils did not justify any judgment that rose to a “convincing” or “probable” level to associate dietary fat with the risk of cancer. In particular, the panel said, “saturated fatty acids . . . have no special relevance to the risk of cancer” (p. 371 of the report; <http://tinyurl.com/WCRF-AICR>).

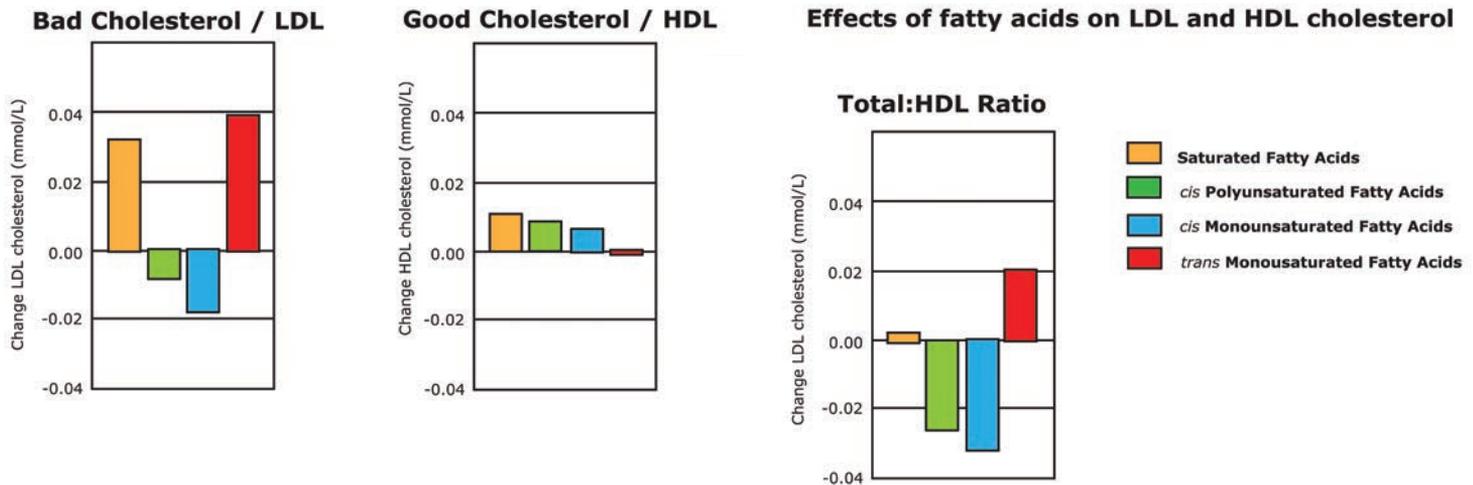
been conducted were inconclusive. As a result, the first Dietary Guidelines Advisory Committee relied almost exclusively on epidemiological data to formulate their recommendations for saturated fat.

In this arena, Keys’ Seven Countries Study loomed large. After all, Keys had shown that people from countries that consumed higher amounts of saturated fats (for example, the United States and Finland) died from heart disease at a greater rate than those who ate less saturated fat (such as Japan and Greece). However, epidemiological studies can only prove correlation, not causation. It’s possible that other diet or lifestyle factors could explain the difference in heart disease deaths. For example, a reanalysis of the Seven Countries Study conducted in 1999 concluded that sugar consumption correlated more strongly than saturated fat intake with heart disease deaths (Menotti, A., *et al.*, *Eur. J. Epidemiol.* 15:507–515, 1999). Populations that ate lower amounts of saturated fat also tended to consume fewer sugary desserts and pastries.

In recent years, the Seven Countries study has been criticized and largely discredited because of troubling methodological problems. Keys apparently included only those countries that would confirm his hypothesis that saturated fat causes heart disease, while excluding countries such as France and Switzerland that consume relatively large amounts of saturated fat yet do not suffer high rates of heart disease deaths. Also, although 12,770 men were asked about their diet and followed for 10 years to see if they died of heart disease, the researchers actually sampled the diets of only about 500 of them. One of the diet surveys in Greece occurred during Lent, when many people were deliberately avoiding animal products. And, finally, although a diet low in saturated fat correlated with fewer deaths from heart disease, saturated fat intake had no effect on total deaths. In other words, people from countries that consumed low amounts of saturated fat had just as high a risk of dying, but they died from other causes.

Other epidemiological studies, as well as controlled clinical trials, have produced conflicting results as to whether saturated fat intake modulates heart disease risk. Pooling data from multiple studies (called a meta-analysis) can help researchers identify patterns when individual studies disagree. In 2010, Krauss and his colleagues performed a meta-analysis of 21 prospective cohort studies of generally healthy people who differed in saturated fat intake (Siri-Tarino, P. W., *et al.*, <http://dx.doi.org/10.3945/ajcn.2009.27725>). The meta-analysis revealed that among the almost 350,000 people included in the 21 studies, a higher intake of saturated fat was not associated with an increased risk of coronary heart disease, stroke, or cardiovascular disease.

More recently, researchers led by Rajiv Chowdhury at the University of Cambridge, in the UK, performed a meta-analysis of 32 observational studies that examined people’s intake of different types of fat and their risk of heart disease (<http://dx.doi.org/10.7326/M13-1788>, 2014). Because study participants sometimes misreport what they eat, the researchers also analyzed 17 studies that actually measured fatty acids circulating in people’s blood as an indication of their diet. In addition, the researchers examined 27 randomized controlled trials of supposedly beneficial fatty acid supplements (e.g., omega-3 polyunsaturated



**Fig. 2.** Effects of fatty acids on LDL and HDL cholesterol. (Left) Saturated and trans fats increase the serum levels of LDL, or “bad,” cholesterol. (Center) Saturated and unsaturated fatty acids increase HDL, or “good,” cholesterol. (Right) Unsaturated fats decrease the ratio of total cholesterol to HDL, indicating a reduction in heart disease risk. Trans fats increase this ratio, while saturated fats do not substantially increase or decrease the ratio. Credit: Gerald McNeill, prepared from data in Mensink, R. P., et al., “Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials.” *Am. J. Clin. Nutr.* 77: 1146-1155, 2003.

fatty acids) given to prevent heart attacks. In total, the meta-analysis included more than 600,000 participants from 18 countries.

Chowdhury and his colleagues found no significant associations between dietary, circulating, or supplementary fatty acids and heart disease risk, with the exception of dietary trans fats, which slightly increased risk in the five studies analyzed. “Current evidence does not clearly support guidelines that encourage high consumption of polyunsaturated fatty acids and low consumption of total saturated fats,” the authors conclude. “Nutritional guidelines on fatty acids and cardiovascular guidelines may require reappraisal to reflect the current evidence.” A revision of the paper retracted its initial conclusions on polyunsaturated acids because of an erroneous analysis of omega-3 polyunsaturated acids, which were indeed associated with cardiovascular benefit. However, the study’s conclusions regarding saturated fat remain unchanged.

Critics of the Krauss and Chowdhury reports point out that the meta-analyses lumped together studies that replaced saturated fat with polyunsaturated fat and those that replaced saturated fat with carbohydrates. “When you look at meta-analyses that actually distinguish between the replacement components, there’s a clear benefit of replacing saturated fat with polyunsaturated fat but not of replacing saturated fat with carbohydrates,” says Alice Lichtenstein, professor of nutrition science and policy at Tufts University and vice-chair of the 2015 Dietary Guidelines Advisory Committee. In other words, saturated fat may be bad for you compared with polyunsaturated fat, but its negative effects could be masked by the fact that carbohydrates are even worse.

Krauss agrees that the overall dietary context is important to consider when interpreting these studies. When people are asked to lower their intake of one nutrient, such as saturated fat, they compensate by increasing their intake of another, such as carbohydrates. “In the context of our findings we tried to explain that saturated fat *per se* cannot easily be connected to adverse effects,” says Krauss. “We’re trying to broaden the discussion from this monomaniacal focus on one single chemical in foods, saturated fat.”

## Time for a change?

With an updated version of the *Dietary Guidelines for Americans* set to be released in the third quarter of 2015, some researchers were hopeful that, given new evidence over the past five years, saturated fat would be at least partially exonerated. However, the Scientific Report of the 2015 Dietary Guidelines Advisory Committee continues to identify saturated fat as a “nutrient of concern for overconsumption” that should be limited to less than 10% of total calories. Although the federal government will have the final say on the *Dietary Guidelines*, it is unlikely that the guidelines will diverge significantly from the scientific report of the committee.

Krauss considers it a good sign that the Dietary Guidelines Advisory Committee hasn’t recommended cutting saturated fat even further, down to the Draconian 5–6% of total calories suggested by the American Heart Association. “Ten percent is a reasonable amount of saturated fat,” he says. “But rather than the notion that people should be



# AOCS



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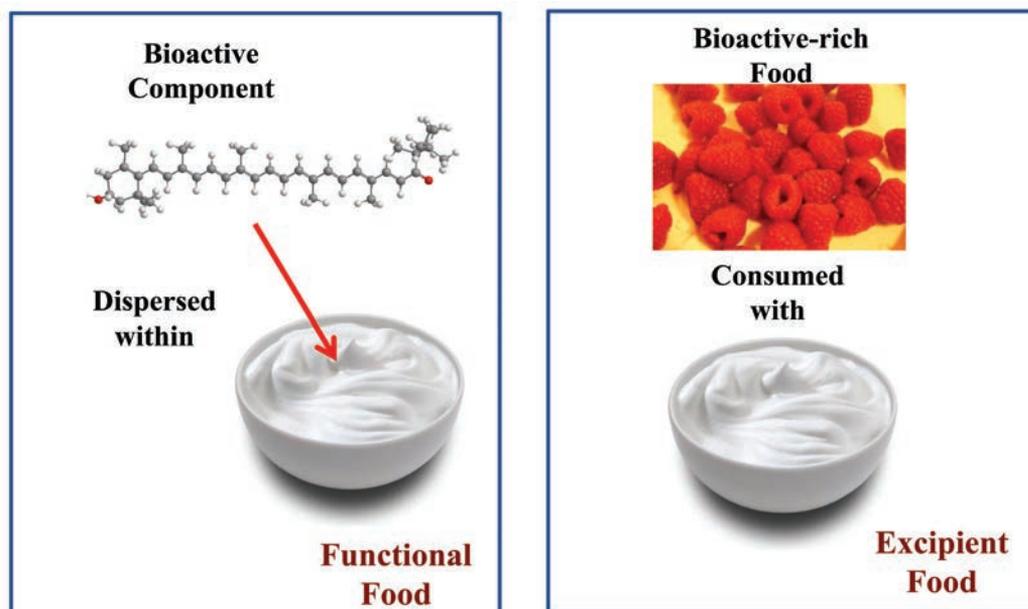
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**FIG. 1.** Schematic diagram of the difference between functional and excipient foods. The bioactive component is usually encapsulated within the food matrix in functional foods, but it is co-ingested with a different food for excipient foods.

# Designing excipient foods to improve oral bioavailability of nutraceuticals

David Julian McClements, Laura Salvia Trujillo, Liqiang Zou, Zipei Zhang, Ruojie Zhang, and Hang Xiao

Natural and processed foods contain a wide variety of bioactive components that have potential health benefits, including vitamins, minerals, and nutraceuticals. Regular consumption of foods containing these bioactive components may therefore help prevent the onset of chronic diseases (such as heart disease, diabetes, hypertension, or cancer) or to improve human wellbeing and performance (such as attention, alertness, stamina, and energy levels).

- Excipient foods increase the bioavailability of bioactive components present in other foods co-ingested with them.
- The structure and composition of excipient emulsions are specifically designed to increase bioavailability by modulating physicochemical and physiological limiting factors.
- The potential health benefits of natural foods, such as fruits and vegetables, can be boosted by consuming them with excipient foods, such as excipient sauces, dressings, creams, yogurts, spreads, dips, pieces, or coatings.

CONTINUED ON NEXT PAGE

**Table 1:** Some potential examples of excipient foods that could be developed to improve the bioavailability of bioactive components in other foods. Adapted from McClements and Xiao 2014.

Food Source	Nutraceuticals	Potential Excipient Foods
<b>Raw Salad</b> - Lettuce, kale, carrot, tomato, peppers ...	Carotenoids	Salad Dressing or Mayonnaise
<b>Cooked vegetables</b> - Carrot, peppers, spinach, kale...	Carotenoids	Cooking Sauce
<b>Nuts and Seeds</b> - Almonds, walnuts, peanuts, sunflower seeds...	Carotenoids, Vitamins, Phytosterols, phytostanols	Edible Coatings or Pieces
<b>Fruits and Berries</b> - Blueberry, strawberry, raspberry, blackberry apple, pear...	Flavonoids, Vitamins	Cream, Ice Cream, Yogurt
<b>Meat and Dairy products</b> - Beef, cheese...	Conjugated Linoleic Acid, Vitamins	Cooking Sauce
<b>Fish</b> - Salmon, menhaden, tuna, mackerel...	$\omega$ -3 Oils	Cooking Sauce
<b>Beverages</b> - Tea, coffee, chocolate...	Polyphenols	Milk, Cream

Nevertheless, the potential health benefits of many bioactive components present within foods are not fully realized because they have low oral bioavailability. In other words, only a small fraction of these healthful components is actually absorbed by the human body in a bioactive form. Consequently, there has been great interest in specifically designing foods to improve the bioavailability of these bioactive components. In general there are two main approaches that can be used based on food matrix design (Fig. 1, page 351).

**Functional foods:** A functional food contains one or more bioactive components incorporated within the food matrix. Some examples of commercially available functional foods are milks fortified with vitamin D, orange juice fortified with calcium, spreads fortified with phytosterols, and yogurts fortified with  $\omega$ -3 fatty acids.

**Excipient foods:** An excipient food consists of a food matrix that is specifically designed to improve the oral bioavailability of bioactive components present in other foods co-ingested with it. Thus, an excipient food may not have any inherent health benefits itself, but it can boost the potential health benefits of other foods, such as fruits and vegetables. Some potential examples of excipient foods that could be designed to increase the bioavailability of bioactive components in other foods are given in Table 1. For example, the bioaccessibility of carotenoids in cooked vegetables may be increased by consuming them with a specifically designed excipient sauce.



$$F = F_L \times F_S \times F_T \times F_A$$

$F_L$  = Fraction Liberated from Food Matrix

$F_S$  = Fraction Solubilized in Gastrointestinal Fluids

$F_T$  = Fraction Not Transformed by Chemical or Biochemical Reactions

$F_A$  = Fraction Absorbed by Epithelium Cells

**FIG. 2.** The oral bioavailability of a bioactive agent depends on various physicochemical and physiological processes occurring with the gastrointestinal tract. Some of the key processes involved are shown schematically.

## Factors limiting bioavailability

The design of food matrices to boost the potential health benefits of bioactive components present within other foods

depends on an understanding of the various factors that normally limit their oral bioavailability (Fig. 2).

**Poor release from food matrix:** Some bioactive components have low bioavailability because they remain trapped within the food matrix. For example, carotenoids may be trapped within plant cell tissues that are not fully broken down within the gastrointestinal tract, and therefore they are not released.

**Low solubility in gastrointestinal fluids:** Many bioactive components have low bioavailability because they have poor solubility in the aqueous gastrointestinal fluids, and are therefore not available for absorption by the epithelium cells. For example, oil-soluble vitamins and carotenoids have a low solubility in gastrointestinal fluids, which limits their bioavailability (Fig. 3, page 354).

**Transformation in gastrointestinal tract:** Certain bioactive components have low bioavailability because they are chemically changed or metabolized within the gastrointestinal tract. For example, curcumin undergoes rapid chemical transformation around neutral pH thereby reducing its bioactivity.

**Poor absorption by epithelium cells:** Some bioactive components have low bioavailability because they are not readily absorbed by the epithelium cells. In other words, they have low intestinal permeability.

Each type of bioactive component has its own unique factors that limit its bioavailability, which depends on its molecular and physical properties, as well as the nature of the surrounding food matrix.

## Designing excipient foods to improve bioavailability

Based on knowledge of the major factors that normally limit bioaccessibility it is possible to design food matrices that boost the potential health benefits of bioactive components in natural and processed foods. Some of the potential strategies that could be used to achieve this goal are highlighted.

**Enhancing release from food matrix:** An excipient food may be designed to contain ingredients that facilitate the breakdown of the food matrix, and therefore the release of the bioactive component. Examples include specific enzymes, chelating agents, surfactants, acids, or bases that disrupt food structures that normally prevent bioactive release.

**Improving solubility in gastrointestinal fluids:** An excipient food may contain ingredients that improve the solubility of the bioactive components within the gastrointestinal fluids. For example, the solubility of highly lipophilic components can be improved by including long chain triglycerides in an excipient food. These triglycerides are converted into free fatty acids and monoglycerides in the small intestine, which form mixed

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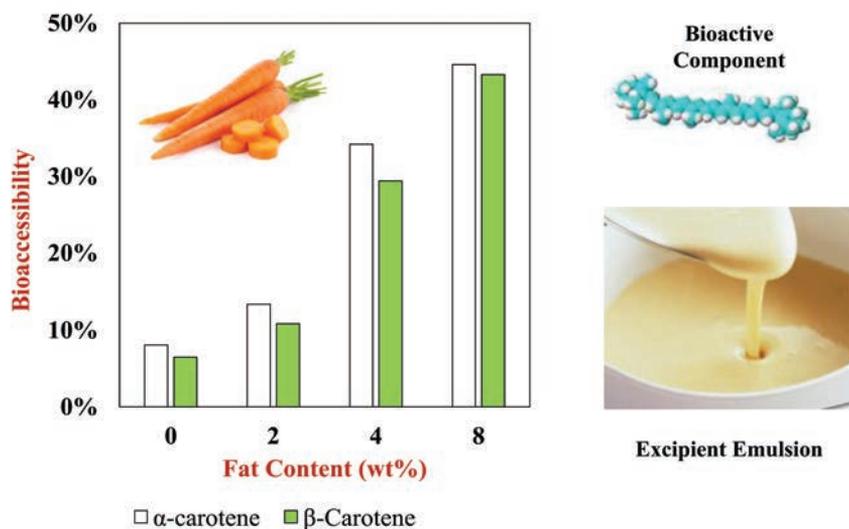
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**FIG. 3.** The bioaccessibility of carotenoids in cooked carrots can be increased by consuming them with an excipient emulsion (such as a cooking sauce). The bioaccessibility increases with increasing fat content because there are more mixed micelles available to solubilize the carotenoids

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micelles capable of solubilizing and transporting lipophilic bioactive components.

**Controlling transformation in gastrointestinal tract:** An excipient food may contain ingredients that modulate the chemical degradation or metabolism of the bioactive components within the gastrointestinal tract. For example, it may contain antioxidants that inhibit oxidation reactions; acids, bases, or buffers that control intestinal pH; or, ingredients that inhibit certain metabolic enzymes.

**Increasing absorption by epithelium cells:** An excipient food may contain ingredients that improve the absorption of the bioactive components by the epithelium cells. For example, it may contain surfactants or biopolymers that increase tight junction or cell wall permeability.

There are many other potential approaches that can be used to improve the bioavailability of bioactive components. The formulation of an effective excipient food will depend on the precise nature of the bioactive component to be boosted, as

well as the type of food that is being designed (e.g., sauce, cream, dressing, dip, and so on).

### Potential examples of excipient foods

In this section, we give some examples of commercial food products that could be specifically designed to have an excipient effect (Table 1).

**Excipient dressings:** Salad dressings and mayonnaise products could be designed to increase the bioavailability of bioactive components naturally present within salads, such as lettuce, kale, spinach, carrots, peppers and tomatoes. Research has already shown that dressings can increase the bioavailability of carotenoids in various types of salad vegetable.

**Excipient sauces:** Cooking sauces could be designed to increase the bioavailability of bioactive components present within cooked vegetables, meats, fish, or spices. For example, our group has recently shown that a model excipient sauce can increase the bioavailability of curcumin from the powdered form.

**Excipient creams:** Creams, such as pouring or whipped creams, could be designed to increase the bioavailability of bioactive components within raw or processed fruits, such as strawberries, raspberries, blueberries, blackberries, or mangoes. They may also be added to fruit smoothies to improve the bioavailability of the bioactives present within the homogenized fruits.

**Excipient yogurts:** Yogurts could also be specifically designed to increase the bioavailability of bioactive components within fruits consumed with them.

*Excipient pieces:* Chocolate chips or other pieces could be designed to increase the bioavailability of bioactives within nuts or grains consumed with them.

Again, the precise design of the composition and structure of the excipient food depends on the major factors limiting the bioavailability of the bioactive components present within the other food source consumed with it.

The principle of designing the composition and structure of food matrices to improve the bioavailability of bioactive agents may lead to the development of a whole new class of foods that can boost the health benefits of natural and processed foods. Research is currently ongoing to create strategies of creating excipient foods that are commercially viable.

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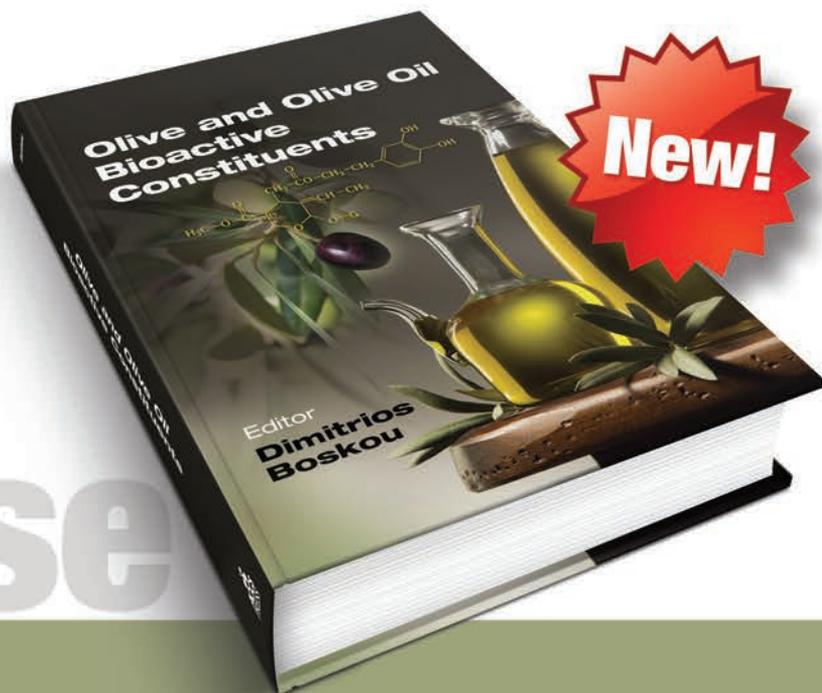
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# New Release



## *Olive and Olive Oil Bioactive Constituents*

Edited by Dimitrios Boskou

2015. Hardback. 422 pages. ISBN 978-1-630670-41-2. Product code 277

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The market is flooded with products posing as elixirs, supplements, functional foods, and olive oil alternatives containing phenols obtained from multiple olive sources. This technically-oriented book will be of value to nutritionists and researchers in the biosciences. It unravels the body of science pertaining to olive minor constituents in relation to new chemical knowledge, technological innovations, and novel methods of recovery, parallel to toxicology, pharmacology, efficacy, doses, claims, and regulation.

Topics include: the biological importance of bioactive compounds present in olive products; developments and innovations to preserve the level of bioactives in table olives and olive oil; and importance of variety, maturity, processing of olives, storage, debittering of olives and table olives as a valuable source of bioactive compounds.

### CONTENTS

- Olive Fruit, Table Olives, and Olive Oil Bioactive Constituents
- Minor Bioactive Olive Oil Components and Health: Key Data for Their Role in Providing Health Benefits in Humans
- Cellular and Molecular Effects of Bioactive Phenolic Compounds in Olives and Olive Oil
- Olive Oil Phenolic Composition as Affected by Geographic Origin, Olive Cultivar, and Cultivation Systems
- Effect of Fruit Maturity on Olive Oil Phenolic Composition and Antioxidant Capacity
- From Drupes to Olive Oil: An Exploration of Olive Key Metabolites
- Research and Innovative Approaches to Obtain Virgin Olive Oils with a Higher Level of Bioactive Constituents
- Table Olives as Sources of Bioactive Compounds
- Bioactive Phenolic Compounds from *Olea Europaea*: A Challenge for Analytical Chemistry
- Analysis of Bioactive Microconstituents in Olives, Olive Oil, and Olive Leaves by NMR Spectroscopy: An Overview of the Last Decade
- Recovery of High Added Value Compounds from Olive Tree Products and Olive Processing Byproducts

## BRIEFS

Cavitation Technologies, Inc. (CTi; Chatsworth, California, USA); announced in April 2015 that the Desmet Ballestra Group, CTi's strategic partner since 2010 and licensee of the company's vegetable oil refining technology, has entered into a new system trial agreement with a 500-metric-ton-per-day sunflower oil refinery in Turkey. This marks the first system trial of CTi's technology in a sunflower oil facility. Desmet Ballestra said it anticipates system installation in the first quarter of 2016, according to a CTi news release.



A special issue of *Food Research International* discusses research gaps in work on how increasing temperature, precipitation, and other effects of climate change may influence food safety. The effects not only aid the growth and survival of plant pathogens but also encourage pests and increase the susceptibility of plants to both pathogens and pests. All of this leads to projected increases in the use of pesticides, causing further environmental contamination. To access the special issue (subscription or payment required), see <http://tinyurl.com/CC-Food-Safety>.

## NEWSMAKERS

Congratulations go to four AOCS members who will be inducted in July 2015 as Fellows of the Institute of Food Technologists (IFT). They are: Michael Eskin, University of Manitoba, Canada; David Julian McClements, University of Massachusetts, USA; Harjinder Singh, Massey University, New Zealand; and Gow-Chin Yen, National Chung Hsing University, Taiwan. The IFT Fellow designation is an honor bestowed upon IFT members by their peers, recognizing exemplary professionalism in the field of food science.



Also celebrating is AOCS member Usha Thiyam-Holländer—chair of the AOCS Lipid Oxidation and Quality Division in 2012–2013—who recently was promoted to associate professor in the Department of Human Nutri-

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# NEWS & NOTEWORTHY



## North American margarine kerfuffle ends

The Great Margarine War has ended. Before the resolution, Québec—with its influential dairy industry—was the last jurisdiction in North America to force margarine manufacturers to make their products either darker or lighter than butter so that retailers and restaurants could not pass off margarine as butter.

The settlement has been a long time coming. It was illegal to sell margarine of any color in Canada until 1948, which was when the Supreme Court revoked a ban that had been on the books since 1886. That was also the year the United States enacted a restrictive tax on margarine. Canadian courts ruled in 1950 that “margarine laws were a provincial jurisdiction,” according to a detailed history

by the Canadian Broadcasting Corp. (CBC; see <http://tinyurl.com/CBC-margarine>).

Fast forward to 2005, when the Supreme Court of Canada ruled that Québec's margarine color requirements would stand until the province decided otherwise. Ten years later, in March 2015, the provincial Cabinet acted to end its role in the butter vs. margarine battle. For its part, Québec's dairy lobby told the CBC that “it has no plans to fight the government's decision to allow Québécois access to yellow margarine.”

## Soy protein health claim approved by Health Canada

Health Canada has approved a health claim linking the consumption of protein-rich soy food to lowering serum cholesterol levels.

CONTINUED ON PAGE 359

# SUSTAINABILITY WATCH

## Flotek and Solazyme team up

Flotek Industries and Solazyme will globally commercialize Flocapso, a drilling fluid additive. In addition, Flotek will market Solazyme's Encapso lubricant—the first commercially available, biodegradable encapsulated algal oil-based lubricant for drilling fluids—in certain Middle Eastern markets. Laboratory and commercial testing, the companies said in a news release, “indicate that the Flocapso additive will allow the use of water-based fluids in wells that previously required more expensive and invasive oil-based products, providing an environmentally superior, more efficient solution to drilling challenges around the globe.”

## Open-Bio: standards for biobased products

Wageningen UR Food & Biobased Research (WURFBR; [www.biobasedeconomy.eu](http://www.biobasedeconomy.eu)) is investigating standards for biobased products as part of the Open-Bio project funded by the European Commission. “There is some ambiguity concerning biobased products that frequently have different functional properties and end-of-life options from petroleum-based products,” said the group in a news release. “To develop and improve these standards, however, misconceptions will first have to be identified and eliminated. Clear standards will help to remove barriers to the introduction of biobased products on the market.”

### LACK OF CLEARLY DEFINED STANDARDS

A variety of biobased products are being developed at present: not only biobased paint, adhesives and solvents but also bioplastics and biopackaging, biobased fertilizers, and additives. Clearly defined standards are lacking for most of these new product groups.

Karin Molenveld, a researcher at WURFBR, explains: “Some standards—the standards for insulation material, for example—are out of date. The standard method for testing insulation values dates from the time when all the insulation materials on the market were manufactured from minerals or fossil-based resources, such as rock wool. Many biobased alternatives have functional properties that are just as good. However, they do not emerge well from the standard functionality tests because their additional, beneficial properties—breathability and vapor permeability, for example—are not included in the current standards. Many companies are put off because of the limitations of the test results.”

### CLARITY ABOUT END-OF-LIFE OPTIONS

There are also misconceptions about the end-of-life options for biobased products, such as compostability, biodegradability, and recyclability.

“When a product is described as ‘compostable’ people tend to assume that it is also biodegradable in, say, a marine environ-

ment,” noted WURFBR researcher Maarten van der Zee. “But that is not always the case . . . [For example], the degradation process in the sea is totally different from the degradation process during composting or in soil.”

Van der Zee takes the view that these and other misconceptions are creating barriers for biobased products. On the one hand, invalid assumptions are drawn from words such as “compostable” and “biodegradable”; on the other hand, businesses such as recycling firms that have to deal with end-of-life options are refusing to accept biobased products. “Because the recycling plants do not know exactly where they stand,” continues Van der Zee, “they are afraid that biobased products will undermine the quality of the recycling stream.” To eliminate these and other misconceptions, Wageningen UR will explore exactly what happens to biobased or compostable packaging when it ends up in a sorting system. The research team will also work on improving the testing methods for determination of biodegradability in different (intended and unintended) end-of-life options such as anaerobic digestion, home composting, and biodegradation in soil and the sea.

### RESEARCH GOALS

In the Open-Bio project, Wageningen UR is researching:

- Obstacles with regard to product functionality
- Methods to determine biobased content as well as biodegradability in home composting conditions and in anaerobic digestion, soil, and the marine environment
- Recycling techniques
- Communication and labelling of biobased products
- Acceptance factors for consumers, the public sector and businesses

## NRC roadmap report

The US National Research Council (NRC), a unit of the National Academy of Sciences, has released a report titled “Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals.” In it, NRC details a series of goals, recommendations, and conclusions regarding expanding the use of industrial biotechnology to transform the sustainability and cost of chemical production.

“The advanced manufacturing of chemicals through biology can help address global challenges related to energy, climate change, sustainable and more productive agriculture, and environmental sustainability,” according to the report. “For example, these processes may help reduce toxic byproducts, reduce greenhouse gas emissions, and lower fossil fuel consumption in chemical production. Lowered costs, increases in production speed, flexibility of manufacturing plants, and increased production capacity are among the many potential benefits.”

The 144-page report may be downloaded free of charge at <http://tinyurl.com/NAP-report>. ■

According to the Soyfoods Association of North America, the soy food market in the United States grew from \$2.28 billion in 1999, when a similar claim went into effect in the United States, to \$4.5 billion in 2013. Brazil, Japan, Malaysia, the Philippines, Indonesia, the United Kingdom, and South Korea have also approved soy health label claims.

Foods that bear the label claim in Canada must:

- Contain at least 6 grams of soy protein per reference amount and per serving of stated size,
- Contain 100 milligrams or fewer of cholesterol per 100 grams of food,
- Meet the conditions for “free of saturated fatty acids” or “low in saturated fatty acids,” and
- Meet the conditions for “source of protein” such that the food has a Reasonable Daily Intake of protein rating of 20 or more.

The format of the primary claim statement is: [Serving size from Nutrition Facts table in metric and common household measures] of (brand name) [name of food] supplies/provides X% of the daily amount of soy protein shown to help reduce/lower cholesterol.

The “daily amount” is 25 g of protein. Additional allowed statements are:

- Soy protein helps reduce/lower cholesterol.
- High cholesterol is a risk factor for heart disease.
- Soy protein helps reduce/lower cholesterol, which is a risk factor for heart disease.

The complete regulation may be found at <http://tinyurl.com/HC-soy>.

## Q&A on FSMA implementation

Joann Givens is the acting regional food and drug director (Central Region) in the US Food and Drug Administration’s (FDA) Office of Regulatory Affairs. Roberta Wagner is the deputy director for regulatory affairs in FDA’s Center for Food Safety and Applied Nutrition. The two women are co-leads of the Phase 2 FSMA Implementation Team Steering Committee, charged with establishing multi-year strategies for gaining widespread industry compliance with the rules mandated by the FDA Food Safety Modernization Act once they become final.

Since January 2013, FDA has proposed seven rules to implement preventive and risk-based standards for the growing, harvesting, packing, processing, and distribution of domestic and imported food for people and animals, from farm through transportation to retail. FDA expects to publish final rules for preventive controls for human and animal food in August 2015, with the final rules for produce safety, and two import-oriented final rules following in October. Further, the agency expects to publish final rules on sanitary transportation and intentional adulteration in March and May 2016, respectively.

Recently, Wagner and Givens shared their insights on what FDA is doing to prepare for FSMA implementation.

### Q: What kind of resources will FDA be providing for industry and federal/state regulators?

*Wagner:* Relative to industry and regulators alike, we are and will continue spending a lot of time writing and issuing industry guidance documents. We recognize that the rules are written in a very flexible way—they are not

tional Sciences at the University of Manitoba in Winnipeg, Canada.



Archer Daniels Midland Co. (ADM; Chicago, Illinois, USA) has appointed John McGowan as president, North American Oilseeds, and Ray Bradbury as president, North American Oils.

In his new position, McGowan will add responsibility for the company’s regional cotton and canola processing businesses to his role as head of the company’s North American soybean operations. In his new role, Bradbury will have oversight of ADM’s North American refineries, its oils portfolio, and biodiesel merchandising.



Solex Thermal Science Inc. (Calgary, Alberta, Canada) has added three new staff members to its Global Oilseeds team. Eric Swenson, an AOCS member since 2008, has been named as an oilseed specialist with the company. Mohamed Abid, who previously was with Ecotech and Alfa Laval, is now oilseed application manager for North America at Solex. Finally, Mario Brugnollo has been hired as technical sales engineer. He brings 12 years of experience in the oilseed industry to his new position.

## IN MEMORIAM

Douglas (Doug) Lopshire, 63, died on Friday, April 17, of complications from Parkinson’s disease.

An AOCS member since 1999, Mr. Lopshire was a graduate of Indiana University (USA) and worked as an auditor with Louis Dreyfus Commodities before transitioning to plant operations. Eventually, he was involved in the engineering of many types of facilities. He had recently retired as plant manager of the Dreyfus facility in Claypool, having moved full-time to Lake James, Indiana.

Mr. Lopshire was active in AOCS as a member of several committees, including those dealing with the budget, financial steering, and business development. He is survived by his wife McGee, his sons Cory and K.C., his grandson Cole, his mother Mary Heemsoth, and his brother Ron.

“There are only a handful of people I have met professionally that I have become close friends with,” said Philip A. Bollheimer, an AOCS member and former president. “After knowing Doug just a few short years my wife and I were taking three-day trips with him and his wife McGee after AOCS meetings out West to visit national parks. We did this three times and had been looking forward to more. Doug was someone I was glad to have known. I love him and will miss him greatly.” ■

CONTINUED ON NEXT PAGE

overly prescriptive—so [industry] will need . . . guidance documents and other materials so that agency expectations are clear. The day-to-day relationship between FDA and state field investigators and inspectors and Center subject matter experts will be critical in meeting our food safety goals while we are developing the aforementioned guidance [documents] and other tools.

*Givens:* We are also going to train the regulators differently. Training on the new standards and how to inspect the industry to determine compliance with these standards will no longer include just one classroom training opportunity. There will be [continual] training for federal and state regulatory personnel, including periodic technical updates regarding industry best practices.

**Q:** Once the rules are final, will there still be opportunities for stakeholders to have a voice in how they are implemented?

*Wagner:* We are going to monitor and evaluate our oversight strategies to determine if we are achieving desired results—for example, widespread industry compliance with the FSMA rules. If we are not achieving the desired results, we will tweak our oversight strategies, perhaps pilot different approaches. I can say we rarely invest resources in assessing whether our imple-

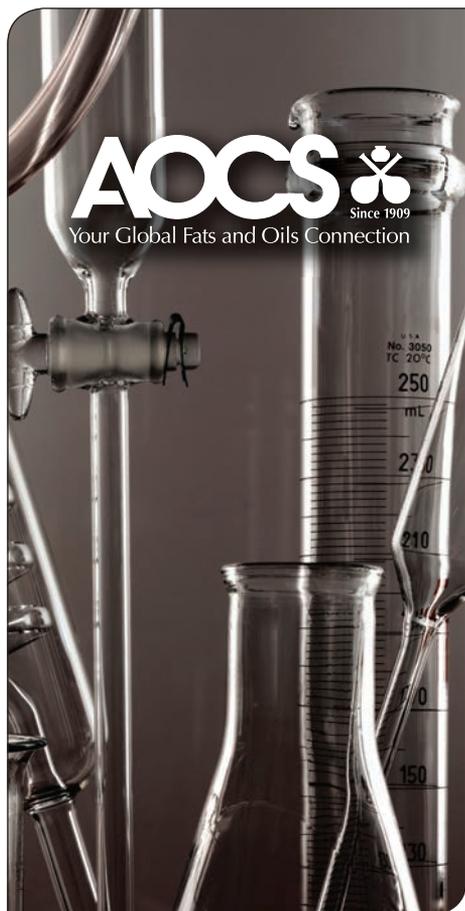
mentation strategies are working. For FDA, this is a very new way of doing business.

## Hershey, IOI Loders Croklaan and shea butter

The Hershey Co. (Hershey, Pennsylvania, USA) will work with Malaysia's IOI Loders Croklaan, a global shea butter supplier, to fund the construction of three warehouses in Northern Ghana that will improve incomes for women shea farmers and, presumably, positively affect their livelihoods.

Hershey and IOI Loders Croklaan will provide funding to StarShea, which commercializes shea butter supplied by its network of rural Ghanaian women, to construct three warehouses, thus allowing the women's cooperative to store shea nuts throughout the season, which will help increase the women's profit for their shea crop.

StarShea will now be able to give women access to much-needed cash earlier in the shea season by buying their shea nuts at a premium at the beginning of the season and storing them in warehouses until they are sold to IOI Loders Croklaan and, ultimately, Hershey. ■



## Supelco/Nicholas Pelick— AOCs Research Award

Since 1964, AOCs has recognized scientists, researchers, engineers, and others for their original research in fats, oils, lipid chemistry, or biochemistry.

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For nomination procedures, deadlines, and full award details, visit: [www.aocs.org/awards](http://www.aocs.org/awards).

*Sponsored by Supelco, a subsidiary of Sigma Aldrich Corp, and Nicholas Pelick, a past president of AOCs.*

# Is Your Lab Prepared for New FSMA Measures?

The new Food Safety Modernization Act (FSMA) measures hold everyone in the food supply chain accountable at every step of production to ensure the safety and purity of food ingredients. Food processors and manufacturers will be required to provide the highest quality results with complete documentation and proof of quality control systems.

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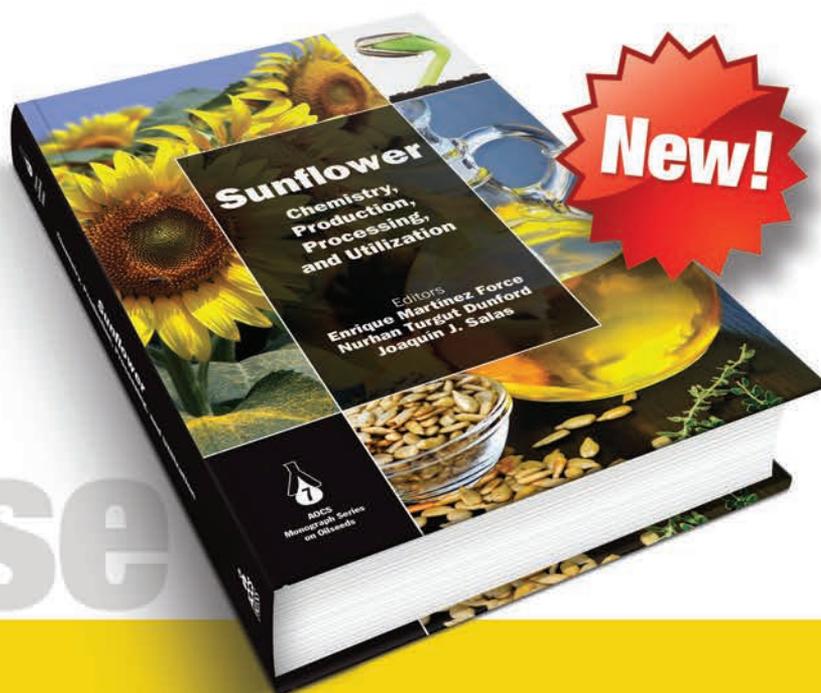
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# New Release



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- Oil Refining
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- South America Perspectives on Sunflower Production and Processing
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- Asia and Australia Perspectives on Sunflower Production and Processing

An article in the *Nikkei Asian Review* (see <http://tinyurl.com/China-fuel>) suggests that China “may finally [be] serious about its biofuel industry.” Among other recent events signaling change was an urgent notice from the National Energy Administration in mid-January 2015 calling for the state oil companies to add biodiesel to their diesel mix. According to the article, if the oil majors did this, annual domestic demand would grow to 8 million metric tons (MMT), up from the 1 MMT currently consumed. However, Sinopec—which together with PetroChina controls 55% of all petrol stations in China—has so far refused to invest in required tanks and infrastructure to support biodiesel blending.

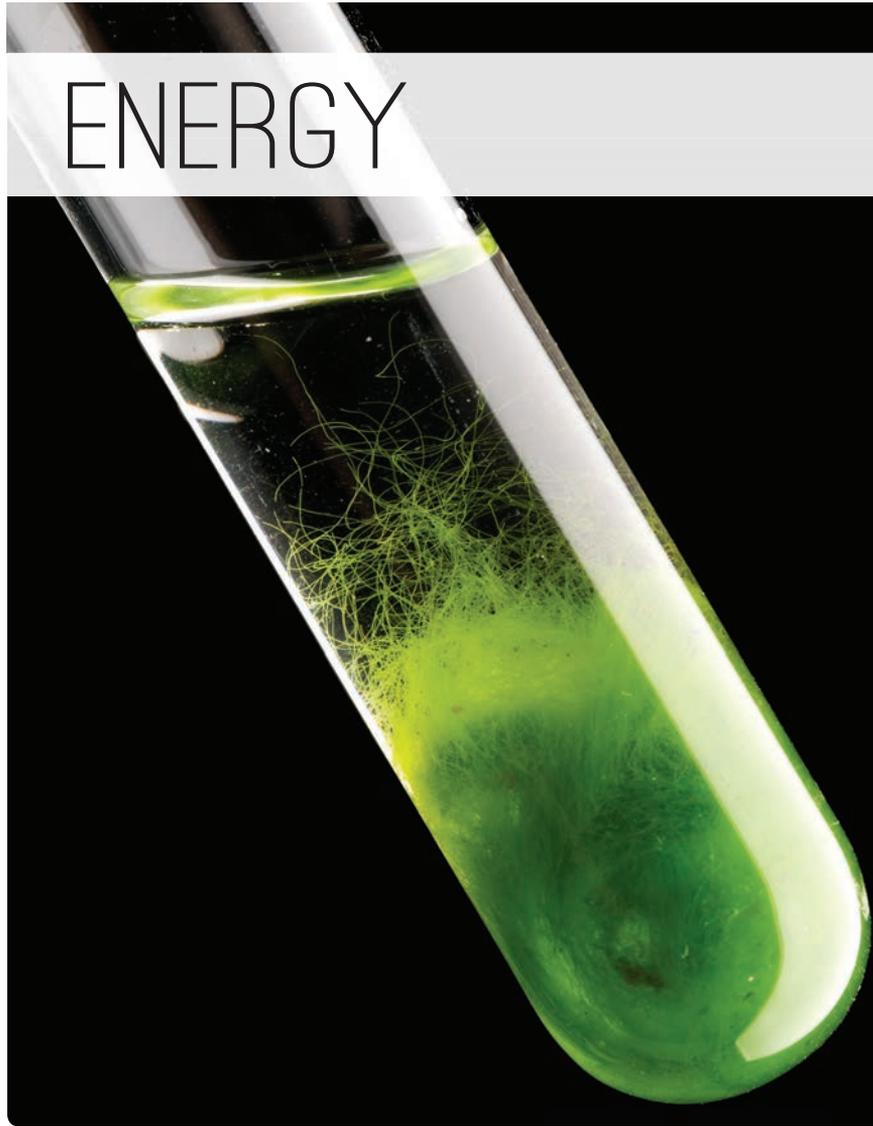


The government of Indonesia is imposing export levies on palm oil to fund biodiesel subsidies, replanting, research and development, and human resources development. As of April 2015, shippers will pay a levy of \$50/metric ton for palm oil and \$30 for processed products, according to BloombergBusiness (see <http://tinyurl.com/Indonesia-levy>). Indonesia increased the mandated amount of palm biodiesel blending in petrochemical-based diesel to 10% in 2014, up from 7.5% in 2013. The mandated amount increased yet again in April 2015, to 15%.



Biofuels produced from pennycress oil (*Thlaspi arvense*) could qualify as biomass-based diesel or advanced biofuel when they are produced using typical fuel production process technologies. So wrote the US Environmental Protection Agency (EPA) in a notice resulting from an analysis of the greenhouse gas (GHG) emissions that come from the production and transport of pennycress oil. According to the analysis, pennycress oil has less than or equal GHG emissions per ton of oil than soybean oil when accounting for crop inputs, crushing, extraction, and direct and indirect land use change. See <http://tinyurl.com/EPA-penny> for the notice; as this issue of *Inform* went to press, the final notice had not yet been posted. ■

# ENERGY



## ABO 2015 Industry Survey results

A survey of the algae industry conducted by the Algae Biomass Organization (ABO; Washington, DC, USA) broadly shows high expectations for continued growth in the sector as well as increased production of a wide range of algae-derived products.

The survey was conducted in February 2015 and included more than 230 responses from companies and individuals involved in directly producing and buying algae or algae-derived products, as well as equipment manufacturers, research laboratories, providers of equipment or materials, government agencies, and service providers.

As in the three previous annual surveys, respondents this year continued their optimism, finding that algae-derived fuels are

likely to be price competitive with fossil fuels by 2020 (75% agreement); that algae based feeds will be commercially available by 2020 (97% agreement); and that algae-based plastics and chemicals will be commercially available in the same timeframe (93% agreement). Further, a majority of producers reported plans to increase production capacity (70% agreement); many expect positive hiring trends through 2015 and beyond.

Overall, ABO said the survey results raise three key points:

- Algal fuels, feeds, plastics, and other products will be price competitive with incumbent products within the decade, if not sooner. Some are price competitive already.
- Production of algal biomass and associated products as well as hiring

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across departments will increase in both the short and the long term.

- Supportive and consistent federal policies will further accelerate production and hiring.

The complete executive summary is available at <http://tinyurl.com/ABO-Survey>.

## Finally: EPA on RFS volumes for 2014, 2015, 2016

In April 2015, the US Environmental Protection Agency (EPA) announced a proposed consent decree in litigation brought against EPA by the American Petroleum Institute and the American Fuel and Petrochemical Manufacturers that would establish the following schedule for issuing Renewable Fuel Standards for 2014 and 2015:

- By June 1, the agency will propose volume requirements for 2015;
- By November 30, EPA will issue final volume requirements for 2014 and 2015 and resolve a pending waiver petition for 2014.

Outside the scope of the consent decree, EPA also commits to:

- Propose the RFS volume requirements for 2016 by June 1, and make them final by November 30;
- Propose and make the RFS biomass-based diesel volume requirement for 2017 final on the same schedule; and
- Re-propose volume requirements for 2014, by June 1, 2015, that reflect the volumes of renewable fuel that were actually used in 2014.

The agency also said it intends to issue a *Federal Register* notice allowing the public to comment on the proposed consent decree. That notice had not been published by the time *Inform* went to press. For more information, see <http://tinyurl.com/EPA-RFS>.

## EPA ALSO WITHDRAWS FINAL RULE ON 6 BIOBASED CHEMICALS

In other agency action, the EPA withdrew a Direct Final Rule for Partial Exemption of Certain Chemical Substances from Reporting Additional Chemical Data on March 30, 2015. The direct final rule, issued in January 2015, would have exempted manufacturers of six biobased diesel chemicals from reporting processing and use information for the compounds under the Chemical Data Reporting (CDR) rule. The EPA decision to withdraw the rule was in response to a single comment posted during the short comment period, according to BRAG (Biobased and Renewable Advocacy Group; Washington, DC, USA).

Kathleen Roberts, BRAG's executive director, stated, "Albeit disappointing, the response is not unexpected given the strict procedures associated with direct final rules. EPA has stated it plans to proceed with a proposed rulemaking to list the chemicals soon and we will urge them to move as quickly as possible. Our hope is EPA can complete the rulemaking process

in time for the next reporting CDR cycle, which starts in June 2016."

Until the new rule is completed, manufacturers of the six affected biobased diesel chemicals should be prepared to submit processing and use information under the CDR in 2016. The CAS registry descriptions of the six chemicals are: fatty acids, tallow, Me esters; fatty acids, C14–18 and C16–18-unsaturated, Me esters; fatty acids, C16-18 and C-18-unsaturated, Me esters; soybean oil, Me esters; fatty acids, canola oil, Me esters; fatty acids, corn oil, Me esters.

## Viridis moves ahead

Viridis Fuels, a biodiesel firm in Oakland, California, USA, has received a \$3.4 million grant from the state of California. Four other companies—Crimson Renewable Energy LP, Community Fuels, AltAir Fuels LLC, and UrbanX Renewables Group Inc.—were also included in the award.

Viridis Fuels plans to build what it calls "America's most visible biodiesel plant," as its project site is situated in the Port of Oakland—the fifth busiest shipping container port in the United States—at the foot of the San Francisco-Oakland Bay Bridge five minutes from San Francisco where more than 225,000 drivers cross on most days, Viridis Fuels President Mario Juarez told *Biodiesel Magazine*.

The company envisions a unique biodiesel plant design, aesthetically speaking, with a sleek wall of LED lighting obscuring the tankage and process equipment. Viridis intends to gain project partnerships with diesel auto manufacturers, displaying their vehicles on the LED wall and illuminating the benefits of both biodiesel fuel and clean diesel technology.

"You need to be creative in today's market," Juarez said.

Viridis Fuels has chosen SRS Engineering to provide a turnkey operation that will utilize low-quality, low-cost feedstock for manufacturing up to 20 million gallons/year (MMgy) of biodiesel and 4 MMgy of technical-grade glycerin. The SRS Engineering biodiesel process at Viridis Fuels will include degumming, bleaching, cold soak filtration, transesterification and esterification, patented resin purification, and removal of sulfur and heavy metals.

Viridis Fuels has a long-term land lease agreement for its project site in the port, one that has already been approved by the California Natural Resources Agency under the California Environmental Quality Act (CEQA).

"Gaining CEQA approval was our biggest environmental hurdle," Juarez said.

He added that the city of Oakland has provided only one franchisee—Oakland Maritime Support Services—approval for the next 35 years to sell fuel to the 10,000 diesel trucks entering the port daily. Juarez said Viridis has signed an agreement to sell up to 5 MMgy of its biodiesel for distribution in the port as B20 (20% biodiesel, 80% petroleum diesel).

With such high truck traffic in and out of the port, it is hoped that biodiesel blends from Viridis Fuels can help clean the air for local residents who, according to Juarez, suffer inordinately high rates of asthma. ■

## BRIEFS

Euromonitor International's (London, United Kingdom) new Passport: Nutrition database allows users to compare nutrients purchased through packaged foods and soft drinks in 54 countries worldwide. A Euromonitor blog post dated April 11, 2015 (<http://tinyurl.com/Med-Euromonitor>) shows how the database can be used to compare dietary patterns in the top eight industrialized nations. The analysis revealed that Germans consume the most calories from packaged food (1,733 Kcal/person/day), while Japanese consumers rank at the bottom of G8 nations for packaged food consumption (1,086 Kcal/person/day). Japan also fares best in sugar consumption from purchased soft drinks (5 grams/person/day), about three times less than Italian consumers and 10 times less than U.S. consumers. Not surprisingly, perhaps, Japan has the lowest rate of obesity (3% of the total population), followed by Italy (11%). The United States and United Kingdom have the highest obesity rates, at 42% and 27%, respectively.



The U.S. Environmental Protection Agency has established a new center to develop alternative approaches for toxicity testing. The Vanderbilt-Pittsburgh Resource for Predictive Toxicology (VPROMPT) is a collaboration between Vanderbilt University (Nashville, Tennessee, USA) and the University of Pittsburgh (Pennsylvania, USA). The new center will receive \$6 million for four years to develop toxicity testing procedures based on three-dimensional human cell cultures, or "organs-on-a-chip." The 3D human cell cultures, which will be wired with different sensors to record how they respond to small concentrations of potentially toxic chemicals, will likely be faster, less costly, and more accurate than traditional toxicology testing procedures involving animals and two-dimensional cell cultures. The researchers will develop four types of organs-on-a-chip: liver cells, fetal cells, mammary gland cells, and cells involved in limb development. ■

## FOOD, HEALTH &amp; NUTRITION



## Vitamin D may keep low-grade prostate cancer in check

For men with less aggressive prostate cancer, taking vitamin D could slow or even reverse tumor growth without the need for surgery or radiation, according to a presentation at the 249th National Meeting & Exposition of the American Chemical Society held in Denver, Colorado, USA March 22–26.

Urologists treat prostate cancer differently depending on the aggressiveness of the tumor. At the time of biopsy, pathologists score prostate tumors on a scale known as the Gleason Grading System. Tumors with Gleason scores of 7 and above are considered aggressive and likely to spread. Men with these tumors typically undergo surgery to remove the prostate gland (prostatectomy) and/or radiation therapy.

However, tumors with Gleason scores below 7 are less aggressive and may cause no symptoms or health problems during the man's lifetime. So in many cases, urologists recommend what's known as active surveillance. "The cure—meaning surgery or radiation—is probably worse than the disease, so

they wait a year and then do another biopsy to see where the patient stands," explains Bruce Hollis at the Medical University of South Carolina, in Charleston, USA, who presented the research.

But some men cannot tolerate having a prostate tumor inside of them, even if it is not aggressive, so they undergo an elective prostatectomy. "They want the tumor out," says Hollis, despite the risk of complications such as erectile dysfunction, urinary incontinence, and infection. A man must wait 60 days after his biopsy before he can undergo a prostatectomy so that inflammation from the biopsy can subside.

Hollis' previous research showed that when men with low-grade prostate cancer took vitamin D supplements for one year, 55% of them experienced improvement in Gleason scores or even complete disappearance of their tumors (*Journal of Clinical Endocrinology and Metabolism*, <http://dx.doi.org/10.1210/jc.2012-1451>, 2012). So Hollis wondered if giving men vitamin D supplements during the 60-day waiting period would improve their tumors.

To find out, Hollis and his colleagues conducted a randomized, controlled clinical

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trial of 37 men undergoing elective prostatectomies. The men were randomly assigned to a group that received 4,000 U of vitamin D per day or to a placebo group that did not receive vitamin D. Sixty days later, the men's prostate glands were removed and analyzed.

Preliminary results from this study indicate that many men in the vitamin D group showed improvements in their prostate tumors after only 60 days of taking the supplement, whereas the tumors in the placebo group stayed the same or worsened. Analysis of gene expression in the tumors revealed dramatic vitamin D-driven changes in the levels of many lipids and proteins, particularly those involved in inflammation. Vitamin D most strongly induced a protein called growth differentiation factor 15 (GDF15) that is thought to reduce inflammation, and is often down regulated in aggressive prostate cancers.

"We don't know yet whether vitamin D treats or prevents prostate cancer," says Hollis. "At the minimum, what it may do is keep lower-grade prostate cancers from going ballistic." Hollis notes that because of these data, vitamin D supplementation for men with low-grade prostate cancer has become a standard practice in the urology division of his university.

## New study reveals metabolic effects of cheese

A new study has uncovered changes in excreted urinary and fecal metabolites after a 2-week diet high in cheese (*Journal of Agricultural and Food Chemistry*, (<http://dx.doi.org/10.1021/jf505878a>, 2015). The results could help explain why the consumption of dairy products, in particular cheese, may reduce cardiovascular disease risk.

Although dairy products are relatively high in saturated fat, some previous studies have indicated that the foods have a modest protective effect against cardiovascular disease. In particular, fermented dairy products such as cheese have been reported to lower LDL cholesterol levels compared with non-fermented dairy such as butter and milk. Some scientists have even speculated that beneficial effects of cheese may explain the French paradox—the observation that people in France have a low rate of heart disease deaths despite a high intake of saturated fat. Indeed, the French are the second-highest consumers of cheese in the world, behind the Greeks.

To gain a better understanding of cheese metabolism, researchers led by Hanne C. Bertram at Aarhus University in Aarslev and Arne Astrup at the University of Copenhagen, both in Denmark, analyzed metabolites excreted by 15 healthy men (ages 18–50) on three different diets. The diets had the same amounts of calories and fat, but were either high in cheese, high in milk (with an equal amount of dairy calcium), or lacking in dairy products other than butter (the control diet). The men consumed each diet for 14 days, with a 14-day washout period in between diets. At the end of each diet, the men provided urine and feces samples.

The researchers used nuclear magnetic resonance (NMR) spectroscopy to identify metabolites in the samples. They found that cheese consumption was associated with a different metabolic response than milk consumption or consumption of the control diet. For example, those who ate cheese had higher fecal levels of butyrate, a compound produced by gut bacteria that can inhibit cholesterol synthesis. Higher butyrate levels were correlated with small but significant reductions in total and LDL cholesterol. Like cheese, milk also showed some potentially beneficial metabolic effects, such as lowering the level of



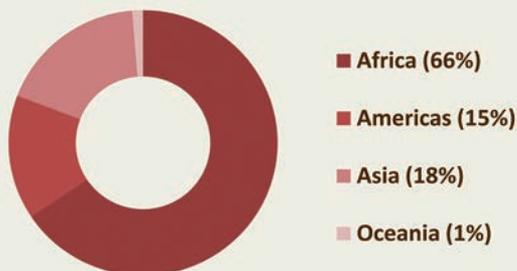
## CHOCOLATE STATS

**4.6** MILLION TONNES  
COCOA BEANS  
PRODUCED  
WORLDWIDE

2013

### COCOA BEAN PRODUCTION

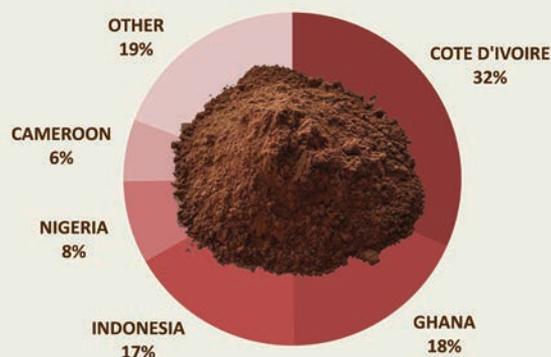
BY REGION (%) Average 1993 – 2013



**CÔTE D'IVOIRE IS THE LARGEST  
PRODUCER OF COCOA BEANS  
1.3 MILLION TONNES**

### WORLD COCOA PRODUCERS

2013



**COCOA PRODUCTION HAS  
INCREASED ALMOST 4X SINCE 1961**

urinary trimethylamine *N*-oxide (TMAO), a possible marker of cardiovascular disease.

“Overall, this metabolomics study suggests that cheese could be an important piece in the French paradox puzzle,” the researchers say. “However, further studies are needed to explore the exact metabolic mechanisms linking cheese consumption, stimulation of the gut microflora, and cholesterol metabolism.”

## Krill oil may be more bioavailable than fish oil

Many people take fish oil supplements in hope of reaping the reported cardiovascular and neurological benefits of omega-3 fatty acids. However, a recent study suggests that omega-3’s in krill oil may be better absorbed by the human body than those in fish oil, but not for the reason researchers suspected (*Lipids in Health and Disease*, <http://dx.doi.org/10.1186/s12944-015-0015-4>, 2015).

Krill oil is derived from shrimp-like crustaceans that feed on algae in deep ocean waters, whereas fish oil comes from fatty fish such as sardines and mackerels. Both fish oil and krill oil contain two omega-3 polyunsaturated fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—widely thought to be beneficial to health, and lacking in the Western diet. Whereas the omega-3’s in fish oil are bound mainly to triglycerides, those in krill oil are attached primarily to phospholipids. Unlike triglycerides, phospholipids are amphiphilic molecules with emulsifying features, which could be expected to enhance their absorption by the human body, although this hypothesis hasn’t been proven experimentally.

To compare the bioavailability of EPA and DHA from different sources, researchers led by Anton Köhler at the University of Munich, in Germany, gave 15 healthy volunteers (7 men, 8 women; mean age, 58 years; all Caucasian) a single dose of either fish oil, krill oil, or krill meal (powdered krill). The amount of each supplement was adjusted to provide about 1,700 mg total omega-3’s. The participants’ diets preceding and during the test days were standardized to preclude dietary differences among subjects. Each participant took all three supplements, waiting 13–29 days between treatments.

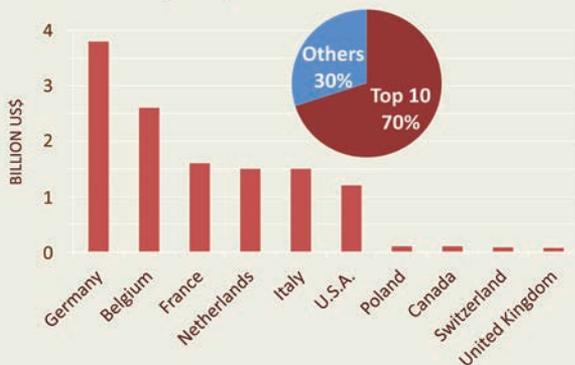
After administering the supplement, the researchers took blood samples from the participants at regular intervals for 72 hours and measured the amounts of EPA and DHA in plasma phospholipid fatty acids. They plotted the amount of detected EPA and DHA versus the time after treatment, and calculated the area under the curve (AUC) as a measure of omega-3 bioavailability.

The largest AUC for EPA and DHA was obtained following krill oil ingestion (mean, 89.08 x h). The mean AUC’s for fish oil (59.15 x h) and krill meal (44.97 x h) were not significantly different. There were large differences in detected EPA and DHA levels among individual study participants; however, 12 of the 15 participants showed the largest AUC for krill oil versus the other supplements.

The researchers do not know why omega-3’s in krill oil were better absorbed than those in krill meal or fish oil. The similar absorption of omega-3’s from fish oil and krill meal argues against the view that phospholipids in krill oil are responsible, since the fat in krill oil and krill meal is identical. Rather, the researchers speculate that differences in the food matrix may explain the superior bioavailability of krill oil. However, they say that these results need to be confirmed in a longer-term trial. ■

## TOP 10 EXPORTERS OF CHOCOLATE PRODUCTS

US DOLLARS (2011)

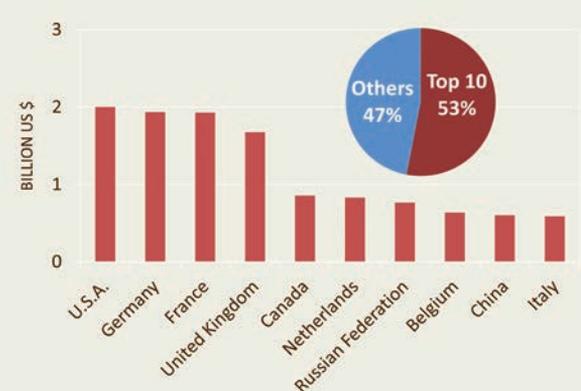


### GERMAN EXPORTS



## TOP 10 IMPORTERS OF CHOCOLATE PRODUCTS

US DOLLARS (2011)



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As a young professional in the oils and fats business, I had heard of AOCS but I really did not know the benefits that AOCS could offer. I was fortunate enough to work for an AOCS member who encouraged me to join. At my first annual meeting I was a bit overwhelmed by all the new faces in the crowd and the myriad of technical topics being presented. However, with each passing year I met more and more people and understood a wider scope of the technology, and my interest level and value from being an AOCS member grew.

I have grown to appreciate the great diversity and reach of AOCS, as well as its challenges. We are a unique organization that has played an important role in many of our careers.

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Timothy Kemper  
AOCS Foundation President 2014–2015  
AOCS President 2013–2014



## BIOTECHNOLOGY

The US Patent and Trademark Office has granted DuPont (Wilmington, Delaware, USA) a patent for sucrose transporter genes for increasing plant seed lipids (Patent Publication Number 8,993,840; awarded March 31, 2015). The invention entails methods for increasing seed oil content by overexpressing the SUT2 and/or SUT4 sucrose transporter genes, in combination with other genes that influence fatty acid accumulation. Increased transport and accumulation of sucrose in plant seed cells drives enhanced lipid synthesis in the seeds.



*Corporate Responsibility Magazine* has named agricultural biotechnology company Monsanto (St. Louis, Missouri, USA) number 41 in its *100 Best Corporate Citizens List* for 2015, according to a news release on the company's website (<http://news.monsanto.com>; April 20, 2015). This is the sixth time that the multinational company has made the magazine's list, which ranks major US companies according to 303 data points of disclosure and performance measures.



The commercial launch of high-oleic soybeans, originally planned for 2014, has been pushed back to 2016 at the earliest because of a lack of global approval for the genetically modified trait, according to an article in *The Western Producer* (<http://www.producer.com/2015/03/high-oleic-soybean-debut-delayed-again>; March 12, 2015). Intended as a replacement for hydrogenated oils, high-oleic soybean oil has higher heat stability and has been touted as more healthful than conventional soybean oil. Farmers in selected areas of some US states already grow high-oleic soybeans, but growers are waiting for global regulatory approval before expanding production. Monsanto's (St. Louis, Missouri, USA) Vistive Gold soybeans await approval in the European Union and China. DuPont Pioneer (Des Moines, Iowa, USA), the other developer of high-oleic soybeans, recently received approval in China for its Plenish soybeans, but is still awaiting approval from the E.U. ■



## GM bacteria make anti-obesity molecule in the gut

Researchers have genetically engineered a strain of bacteria that produces the precursor of a hunger-suppressing lipid. When mice drank water containing the bacteria, they ate less, had lower body fat, and showed reduced insulin resistance, according to a presentation at the 249<sup>th</sup> National Meeting & Exposition of the American Chemical Society, which took place in Denver, Colorado, USA, March 22–26.

Sean Davies at Vanderbilt University in Nashville, Tennessee, USA, and his colleagues introduced a gene to the probiotic intestinal bacterium *E. coli* Nissile 1917 that caused the bacteria to produce and secrete large amounts of *N*-acyl-phosphatidylethanolamines (NAPEs). NAPEs are normally produced in the small intestine after a meal and then rapidly converted by an enzyme

into *N*-acyl-ethanolamines (NAEs), which are potent appetite-suppressing lipids.

The researchers added the bacteria to the drinking water of mice that, when fed a high-fat diet (60% of calories from fat), develop obesity, signs of diabetes, and fatty livers. Compared with control mice that received non-NAPE-producing bacteria, mice that drank the NAPE-making microbes for 8 weeks gained 15% less weight. The treated mice also showed fewer signs of insulin resistance and fatty livers than control mice. The main explanation for these effects was that the treated mice ate less food than controls because of the appetite-suppressing lipids they produced. The beneficial effects persisted for up to 12 weeks after the mice stopped receiving the bacteria. These results were reported late last year in *The Journal of Clinical Investigation* (<http://dx.doi.org/10.1172/JCI72517>, 2014).

In subsequent research presented at the meeting, Davies and his coworkers showed that mice lacking the enzyme to convert

CONTINUED ON NEXT PAGE

NAPes into NAEs did not benefit from NAPE-producing bacteria, so the researchers gave the mice NAE-making bacteria instead. Because some people might not make sufficient amounts of the enzyme that converts NAPes to NAEs, “This suggests that it might be best to use NAE-making bacteria in eventual clinical trials,” said Davies. “We think that this would work very well in humans.”

However, Davies admits that much work remains to be done before the bacteria could be tested as an obesity treatment for people. First, the researchers need to establish the long-term safety of the treatment. Also, the team plans to genetically cripple the bacteria so that they cannot survive outside of the gut. Otherwise, people may unintentionally transfer the bacteria to others by fecal exposure, which could be harmful to some people who do not need to lose weight, for example, the very young, very old, or those with certain diseases.

But if these hurdles can be overcome, Davies says that the bacteria may be a more desirable and effective long-term treatment for obesity than lifestyle changes, which are often difficult to maintain, or weight-loss drugs, which need to be taken on a daily basis.

## Making photosynthesis more efficient

Improvements in crop photosynthesis are needed to feed an estimated 9.5 billion people on planet earth by the year 2050, according to a recent review in *Cell* (<http://dx.doi.org/10.1016/j.cell.2015.03.019>, 2015). Now is the time to invest in high-performance computing, genetic engineering, and other approaches to improve photosynthetic efficiency, say Stephen P. Long and Amy Marshall-Colon at the University of Illinois, Urbana, USA, and their co-author Xin-Guang Zhu at CAS-MPG Partner Institute of Computational Biology in Shanghai, China.

Current models predict that, without major improvements in crop yield, population growth and increased urbanization will lead to serious global food shortages by 2050. Modern advances in biotechnology and agronomy have greatly increased crop

yields, but Long and his colleagues posit that these types of improvements have nearly reached their full potential. For example, scientists have genetically engineered some crop varieties to increase their harvest index, or proportion of the plant's biomass that is in the harvested product, such as the soybean. However, further major improvements in harvest index are unlikely if the plants are to retain their supporting structural components, such as stems and pod casings.

In contrast, decades of crop selective breeding and bioengineering have failed to significantly improve the process of photosynthesis, which is much less efficient than its theoretical limit. If plants could more efficiently harness the power of sunlight to make food, crop yield would rise substantially. The authors argue that improving crop photosynthetic efficiency has only recently become a possibility because of three factors: an enhanced understanding of photosynthesis and its key proteins, the emergence of high-performance computing to model permutations in photosynthetic pathways, and advances in genetic engineering.

The researchers outline different strategies for improving crop photosynthesis, some of which have already demonstrated potential in the lab or in computer models. For instance, pigments in plants primarily capture and utilize energy from the visible spectrum of sunlight, which comprises less than half of the available solar energy. On the other hand, pigments from some photosynthetic bacteria and algae can capture longer wavelengths of light. Genetically introducing these pigments to crops could boost their photosynthetic efficiencies by 10–30%.

Another strategy involves substituting the plant photosynthetic enzyme Rubisco for other forms of the enzyme that are better adapted to today's atmospheric level of carbon dioxide, which is approximately twice that present during much of plant evolution. Computer simulations predict that an increase in Rubisco's catalytic efficiency, at the expense of reduced specificity for carbon dioxide (acceptable because carbon dioxide in the air is more plentiful now than in the past), could increase photosynthetic efficiency by up to 30%.

The authors urge that now is the time to invest in research aimed at improving crop photosynthesis. “Given the 20 to 30 year gap between demonstration of innovative solutions at the experimental level and provision of seed to farmers, the need to

# AOCS MEETING WATCH

October 27–30, 2015. SODEOPEC2015, Hyatt Regency Miami, Miami, Florida, USA. <http://sodeopec.aocs.org>

November 12–13, 2015. AOCS Oils and Fats World Market Update 2015, The Convention Centre Dublin, Dublin, Ireland. <http://worldmarket.aocs.org>

May 1–4, 2016. 107th AOCS Annual Meeting & Expo, Calvin L. Rampton Salt Palace Convention Center, Salt Lake City, Utah, USA.

For in-depth details on these and other upcoming meetings, visit <http://aocs.org/meetings> or contact the AOCS Meetings Department (email: [meetings@aocs.org](mailto:meetings@aocs.org); phone: +1 217-693-4821; fax: +1 217-693-4865).

Also, be sure to visit AOCS' online listing of industry events and meetings at <http://tinyurl.com/industry-calendar>. Sponsoring organizations can submit information about their events to the web-based calendar by clicking a link and completing a web form. Submission is free. No third-party submissions, please. If you have any questions or comments, please contact Liz McMillen at [liz.mcmillen@aocs.org](mailto:liz.mcmillen@aocs.org).

bridge and accelerate the gap between molecular engineering and practical crop breeding to achieve higher yields cannot be postponed, especially considering the forecast situation for 2050,” they write.

## Newly discovered cacao gene determines melting point of cocoa butter

The discovery of a gene that determines the melting point of cocoa butter may have wide-ranging biotech applications, from creating hardier varieties of cacao plants to producing specialty chocolates, according to a recent paper in *Frontiers in Plant Science* (<http://dx.doi.org/10.3389/fpls.2015.00239>, 2015). During cocoa seed development, the enzyme encoded by the gene alters the fatty acid composition of cocoa butter in the seed.

Farmers harvest seeds from the cacao tree, *Theobroma cacao L.*, about 20 weeks after pollination. At that time, the seeds contain about 50% total lipids—known as cocoa butter—that consist of almost equal amounts of palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1<sup>n-9</sup>). This fatty acid composition gives cocoa butter a melting temperature very close to the temperature of the human body, conferring the “melt-in-your-mouth” feel of chocolate and the creamy texture of personal care products that contain cocoa butter.

In other plant species, an enzyme called stearoyl-acyl carrier protein desaturase (SAD) is known to convert stearic acid in acyl carrier proteins to oleic acid, thereby controlling the ratio of saturated and unsaturated fatty acids in the plant tissue. In previous work, Mark J. Guiltinan at The Pennsylvania State University, in University Park, USA, and his collaborators from the International Cocoa Genome Consortium sequenced the entire genome of the cacao plant and identified a family of eight possible SAD isoforms (*Nature Genetics*, <http://dx.doi.org/10.1038/ng.736>, 2011).

In the new study, Guiltinan and his coworkers characterized the cacao SAD gene family in detail. One of the eight isoforms, *TcSAD1*, was expressed across all plant tissues, and its expression pattern in seeds correlated with the dramatic changes in fatty acid composition that occur during seed maturation. Therefore, the researchers concluded that the *TcSAD1* gene is primarily involved in the synthesis of cocoa butter and the determination of its melting point.

By producing varieties of cacao trees with different levels of *TcSAD1* expression, scientists might be able to control the composition and melting point of cocoa butter, which could aid in the production of chocolate with specific textures and specialty applications, as well as new cosmetic and pharmaceutical products. “The ‘snap’ and ‘melt’ of chocolate are two very important textural features that determine the appeal of chocolate to consumers, and having new varieties of the cacao plant that produce butter with different melting points would be a valuable resource to control those characteristics,” says Guiltinan. In addition, because SADs in other species play roles in plant defense, the gene might also be manipulated to produce varieties of cacao that are resistant to pathogens. ■



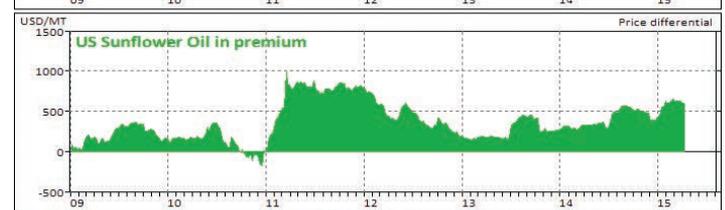
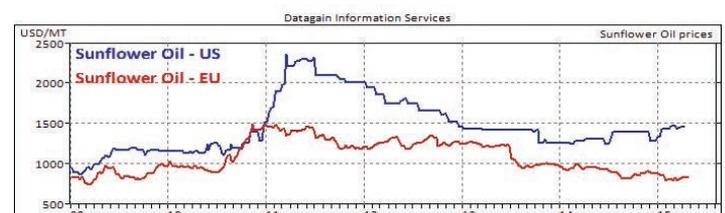
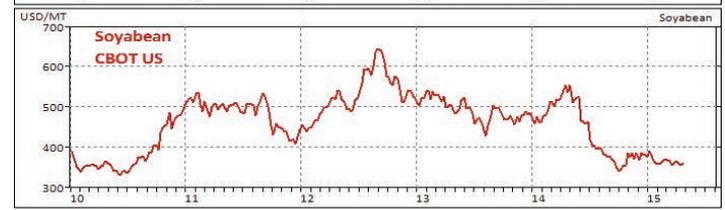
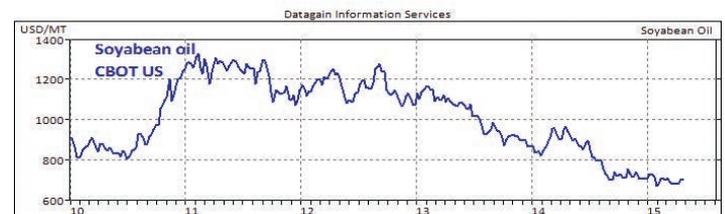
## STATISTICAL ANALYSIS FROM MINTEC

Lorraine Hudson

Global soybean and soybean oil prices have fallen in the first half of the year due to continuing good supply. Global soybean production for 2014/15 is estimated at 315.4 million metric tons (MMT), up 10% year-on-year, and soybean oil production is estimated at 47.3 MMT, up 5% year-on-year. Soybean crush is also estimated to increase to 253.9 MMT, up 5% year-on-year. Global soybean oil consumption is estimated at 46.9 MMT, up 4% year-on-year. US soybean oil production is estimated at 9.3 MMT, up 3% year-on-year.

Production of soybean oil in China, the world’s largest producer, is estimated to rise to 13.3 MMT, up 8% year-on-year. Domestic consumption is estimated to rise by 4% year-on-year to 14.2 MMT. As the rise in production has outstripped the rise in consumption there is a reduced need for imports. Chinese imports are estimated to fall to 1.0 MMT, down from 1.3 MMT in 2013/14. However, production in India, the largest importer of soybean oil, is estimated to fall by 10% to 1.3 MMT. India’s consumption is estimated to rise 3% year-on-year to 3.4 MMT, therefore imports into India are estimated to rise to 2.0 MMT, up 9% year-on-year.

EU sunflower oil prices rose slightly during March and April due to forecasts for lower production. EU production in 2014/15 is expected to fall 2% year-on-year to 3.1 MMT. Global sunflower oil production is also forecast to fall to 15.3 MMT, down 5% year-on-year. Global consumption is estimated at 14.8 MMT, up 2% year-on-year but ending stocks are estimated at 1.8 MMT, down 9% year-on-year.





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The US Food and Drug Administration has started cracking down on cosmetics that make drug claims. Since November 2014, the agency has sent five warning letters and issued a document that explains to consumers the types of claims cosmetics are allowed to make (<http://tinyurl.com/pvw3k6>). Under U.S. federal law, cosmetics are allowed to make claims that they enhance beauty or attractiveness or alter appearance. However, products that claim to treat a disease or affect the structure or function of the body must be evaluated and approved by the FDA prior to entering the market. The chastised companies were instructed to either revise their product labels or submit a New Drug Application to the FDA.



One in five skin creams from Europe, the United States, and Asia with “squalene” in their ingredients list still contain shark oil, according to a recent study by the non-profit Bloom Association (Paris, France). Shark squalene was found mainly in Asian skin creams, with more than half of the tested creams containing the compound. Until recently, squalene from shark liver oil was a common moisturizer in cosmetics. However, environmental concerns have led many cosmetic companies to switch to squalene from olive oil, even though the plant version is 30% more expensive. According to Laure Ducos, lead author of the study, some suppliers may be deceiving cosmetics companies by claiming that shark-derived squalene comes from olive oil (<http://tinyurl.com/m94gu7m>).



On April 16, 2015, new maximum concentration limits for propylparaben and butylparaben sold in the European Union took effect. The new limits reduce the maximum allowed concentration of the preservatives to 0.14% when used individually or together. Previously, the preservatives could be used at 0.4% individually or 0.8% when mixed. The new rules also ban the use of the parabens in leave-on products for the diaper area of children under age 3. Products placed on the market after April 15 must comply with the new limits, but existing stocks may be sold until October 16, 2015. ■

## HOME & PERSONAL CARE



The antimicrobial activity of a “natural” preservative made from radish kimchi (shown here) was traced to synthetic ingredients.

### Kimchi-based preservative is not so natural

Consumer preferences have driven cosmetic companies to seek natural alternatives to synthetic preservatives such as parabens. However, a recent report in the *Journal of Agricultural and Food Chemistry* indicates that a supposedly natural preservative made from fermented radish is contaminated with two synthetic antimicrobial ingredients (<http://dx.doi.org/10.1021/jf5063588>, 2015).

Kimchi is a traditional Korean dish made by fermenting vegetables such as radishes with lactic acid bacteria. Bacteria used in the fermentation, especially *Leuconostoc kimchii*, are potential sources of antimicrobial peptides called bacteriocins. In 2010, The Estée Lauder Companies (New York City, USA) patented a mixture of *L. kimchii* fermentation products as a preservative for cosmetics (U.S. Patent 2010/0129305).

Although the patent claims that the mixture has activity against Gram-positive and –negative bacteria, as well as fungi, no

known antimicrobial peptide from *L. kimchii* exhibits such broad-spectrum antimicrobial activity. So researchers led by John C. Vederas at the University of Alberta (Edmonton, Canada) decided to characterize the antimicrobial components in a commercial *Leuconostoc*/radish root ferment filtrate (LRRFF).

The researchers first confirmed that the commercial LRRFF (marketed as Leucidal Liquid; Active Micro Technologies, Lincoln, North Carolina, USA) inhibited the growth of both Gram-negative and –positive bacteria. Suspiciously, however, the LRRFF retained its antimicrobial activity after treatment with heat or a protease, conditions that would typically degrade antimicrobial peptides.

When the team separated the LRRFF into different fractions and analyzed the components, they identified two chemical preservatives: salicylic acid, which was the primary agent in LRRFF with activity against Gram-negative bacteria; and a didecyldimethylammonium salt, which was responsible for the Gram-positive activity. Carbon dating revealed that the two preservatives were

CONTINUED ON NEXT PAGE

derived from petroleum-based precursors rather than a recent fermentation event, indicating a synthetic origin. No antimicrobial peptides were detected.

Wilson Lee, Director of Research and Development at Estée Lauder Co. and co-author of the kimchi patent, stands by the antimicrobial activity of fermented radish extract. However, he told *Inform* that Estée Lauder does not use the particular LRRFF source tested in the study. Lee notes that kimchi must be fully fermented to produce antimicrobial peptides, and that perhaps the commercial source was not fermented sufficiently. “Our kimchi is fermented at least 6 months before extraction,” he says.

## 3D mapping of the human skin surface

Skin, the largest and most exposed organ of the human body, is composed of molecules derived from skin cells, microbes, personal care products, and the environment. Now, for the first time, researchers have characterized the skin surface distributions of molecules and microbes across the entire human body (*Proceedings of the National Academy of Sciences U. S. A.*, <http://dx.doi.org/10.1073/pnas.1424409112>, 2015). The technique may reveal new insights into the complex molecular interactions that take place on the skin surface.

In recent years, scientists have begun to appreciate the important contributions of the microbiome—commensal microorganisms living on or within the body—to health and disease. However, very little is known about how molecules on the skin surface determine the distribution of microbial species, how microbes chemically modify skin molecules to alter the local environment, or how personal care products influence these interactions.

So an international team of researchers led by Pieter C. Dorrestein, Nuno Bandeira, and Rob Knight at the University of California, San Diego, USA, and Theodore Alexandrov at the University of Bremen, in Germany, developed an approach to visualize the chemical and microbial composition of human skin through the creation of 3D topographical maps. The researchers swabbed the skin surfaces of one male and one female volunteer at 400 different body sites. They then analyzed chemicals at each site using mass spectrometry and identified microbes by sequencing 16S ribosomal RNA. The team used MATLAB software to construct 3D models of the human body to visualize the data obtained at each sampling site, with a color scale corresponding to the amount of molecule or microbe detected.

By comparing mass spectra to those of known skin molecules, bacterial products, and personal care ingredients, the researchers were able to identify about 20% of the detected chemicals. Although the volunteers did not shower or apply personal care products for 3 days prior to the sample collection, a large portion of the identified molecules matched beauty products or cosmetic ingredients, such as surfactants, polymeric substances, and sunscreens. The researchers say that this finding reflects “the lasting impact of our beauty and hygiene products on the molecular composition of the outermost layer of skin that is exposed to the environment.”

The researchers detected 36 phyla of bacteria among the 400 body sites, with the most common being *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. Topographical mapping showed that different microbes localized to different areas of the body. For example, the family *Staphylococcaceae* was found in moist areas, such as the feet of both volunteers, under the female’s breast and neck, and around the male’s nose. In contrast, the genus *Propionibacterium* was found in regions with a high density of sebaceous glands, such as the head, face, upper back, and chest.

Some molecules spatially correlated with specific bacteria. For instance, the localization of oleic acid and palmitic acid mirrored that of *Propionibacterium*. The researchers hypothesize that the bacteria produced these lipids by hydrolyzing human acylglycerides.

This study establishes the feasibility of 3D molecular mapping of the human skin surface and lays the foundation for future studies involving additional volunteers. As more human skin maps become available, scientists could gain a better understanding of how skin chemistry changes in response to microbes, personal care products, and environmental factors.

## Sizing up down-the-drain chemicals

Every day people discharge home and personal care products, pharmaceuticals, and other chemicals to waterways by bathing, washing clothes, pouring unwanted products down the sink, or using the toilet. In a recent study reported in *Science of the Total Environment*, Katherine E. Kapo and her colleagues at Waterborne Environmental, Inc. (Leesburg, Virginia, USA) teamed up with Procter & Gamble (Cincinnati, Ohio, USA) scientists to estimate levels of down-the-drain chemicals in rivers and streams on a broad geographical scale (<http://dx.doi.org/10.1016/j.scitotenv.2015.02.105>, 2015).

The ecological risk posed by chemicals discharged from wastewater treatment plants is strongly related to the dilution level of the chemical, which varies by geography, water flow rate, and the level of the chemical already present in the waterway from upstream wastewater treatment plants. Also, the rate at which the chemical becomes degraded or volatilized in water (in other words, the in-stream decay rate) influences its dilution level.

When assessing ecological risk over large geographical scales (for example, the national level), it is more informative to provide ranges of exposure levels, rather than a single average value or worst-case scenario. So Kapo and her colleagues used a web-based tool developed by the American Cleaning Institute (iSTREEM; Washington, DC, USA) to estimate distributions of dilution factors and per capita wastewater generation at the national level. The analysis was not specific to any one chemical, but was instead applicable to all down-the-drain chemicals.

The iSTREEM model incorporates data from more than 10,000 wastewater treatment plants and 1,700 intake sites of drinking water treatment facilities, covering more than 200,000 river miles. Using these data, iSTREEM can provide an estimated concentration of a chemical at a particular sampling point along a waterway.

The researchers used iSTREEM to calculate dilution factors—defined as the ratio of receiving water to effluent—of down-the-drain chemicals under different scenarios. Modeling chemicals with a range of decay rates from zero decay to a 1-h half-life, iSTREEM estimated median dilution factors for wastewater treatment plant mixing zones ranging from 132 to 609 at a mean flow rate and 5 to 25 at a low flow rate. At drinking water intake sites, the median dilution factors ranged from 146 to  $2 \times 10^7$ , depending on the in-stream decay rate of the chemical. For wastewater treatment facilities with effluent primarily from

domestic sources, the median per capita wastewater generation was 399 L/cap/day.

According to the authors, the estimates derived in this study can be used to conduct probabilistic exposure assessments for down-the-drain chemicals at the national level. The dilution factor distributions can be used to evaluate exposure and risk for a variety of chemicals, from rapidly degrading to highly persistent, at drinking water intake sites and wastewater treatment plant mixing zones with both mean and low flow. ■

## Fat controversy (cont. from 349)

fanatically adding up the saturated fats in their diet and figuring out how much they should be eating, people should be more concerned about their overall dietary pattern and the types of foods they choose.”

He notes that the type of food in which the saturated fat is contained, or the “food matrix,” may influence heart disease risk. For example, some studies suggest that fermented dairy products such as cheese—often avoided because of its high saturated fat content—may contain specific saturated fatty acids that, in the context of other components in the cheese, may actually lower the risk of heart disease and type 2 diabetes.

With regard to food choices, Krauss was dismayed when press coverage of his 2010 meta-analysis tended toward sensational headlines proclaiming it’s okay to load up on sticks of butter and triple burgers with cheese. “That’s not the message,” says Krauss. “The message is to make food choices that are balanced in the overall diet and not to consider any given food the salvation of health or the kiss of death.”

Teicholz believes that, in light of recent evidence, the *Dietary Guidelines* should ditch limits on saturated fat, but she thinks that change is unlikely to occur. “We’re in the third generation of scientists who believe fat, and especially saturated fat, is bad for health,” she says. “The bias is deeply entrenched, and it’s very hard to reverse that.”

She points out that the Scientific Report of the 2015 Dietary Guidelines Advisory Committee states that a healthy dietary pattern should be one low in lean meat (both for dietary and environmental reasons). “They’re recommending an essentially meatless diet for all Americans, and that’s based primarily on this saturated fat concept,” she says. Not only is a vegetarian diet impractical for many, but Teicholz worries that people will get the erroneous message that it’s more healthful to replace meats with carbohydrates such as rice, pasta, or bread. Counter to popular perception, carbohydrates contribute more to obesity and cardiovascular disease risk than saturated fat, she says.

Gerald McNeill, vice president of research and development at IOI Loders Croklaan, an edible oil supplier with US headquarters in Channahon, Illinois, notes that calls to further limit saturated fat would necessitate drastic changes in the US diet. “Seventy-five percent of all dietary satu-

rated fat is contained in meats, dairy products, and eggs,” he says. “To reduce saturated fat to 5% of total calories, a reduction of approximately 65% of these foods in the diet is required.” McNeill notes that, in addition to the decimation of the meat and dairy industries, the near-elimination of these foods would deprive people of important sources of vitamins, minerals, proteins, and fats. “Implementation of such a recommendation could plunge the dietary status of the nation into the dark ages,” he predicts.

Teicholz questions the wisdom of replacing saturated fats with polyunsaturated fats, as recommended by the *Dietary Guidelines*. Although polyunsaturated fats do lower LDL cholesterol and total:HDL cholesterol, their double bonds make them more prone to oxidation than saturated fats, especially when heated during food preparation. Aldehydes produced from polyunsaturated fats can react with DNA, proteins, and lipids in the body, possibly interfering with their functions (Grootveld, M., *et al.*, *Inform* 25:614–624, 2014). Some studies suggest that omega-6 polyunsaturated fatty acids, contained in many vegetable oils, increase inflammation and even promote diseases such as cancer, cardiovascular disease, and type 2 diabetes (reviewed in Lawrence, G. D., <http://dx.doi.org/10.3945/an.113.003657>, 2013). Teicholz calls the massive increase in vegetable oil consumption over the past century “the biggest change in the American diet”—a dietary experiment for which we may not yet appreciate the consequences.

In a promising sign that the Dietary Guidelines Advisory Committee is, in some instances, willing to change its course, the 2015 Committee recommended withdrawing its long-standing warnings about dietary cholesterol. This reversal comes after years of studies showing that eating foods rich in cholesterol, like eggs, doesn’t actually raise cholesterol levels in the blood or contribute to heart disease. Perhaps in 2020 saturated fat will join cholesterol in exoneration, but the pervasive messages that these dietary components are bad for health will likely take decades to erase in the minds of nutritionists and the general public alike.

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# Codex meets in Melaka

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

## John Hancock

“Codex Alimentarius is about safe, good food for everyone—everywhere.” This is a laudable mission statement for the collection of voluntary international standards codified by the United Nations’ Codex Alimentarius Commission, but further reading finds that it is also about providing international food standards, guidelines, and codes of practice that “contribute to the safety, quality, and fairness of the international food trade.” Delegates to the more recent meetings of the Codex Committee on Fats and Oils (CCFO) might feel that it is losing sight of this latter goal and concentrating more strongly on food safety, to the detriment of the trade.

The CCFO met in Melaka, Malaysia, February 9–13, 2015, at the invitation of the Malaysian government, which currently holds the secretariat of the committee. There were some new aspects to this meeting. First, there were two physical meetings of the electronic working groups (eWG) that had been formed at the previous meeting to prepare papers to facilitate the discussion within the plenary sessions. Second, all the trade associations that have observer status at CCFO were invited to submit a report on the topics that were of interest to them.

A major topic for the last 15 years of CCFO meetings has been the Codex Recommended International Code of

Practice for the Storage and Transport of Edible Fats and Oils in Bulk (CAC/RCP 36; see <http://tinyurl.com/CAC-RCP-36>). This document includes banned and acceptable lists of previous cargoes for ships involved in the carriage of oils by sea, with the aim of reducing the risk to consumers from any contamination that may occur. With the introduction by the European Union in the mid-1990s and by China in 2013 of legislation requiring the use of the acceptable list only, the contract-issuing trade associations are keen that Codex maintains the concept of two lists, thus mirroring the vast majority of international trade. Although Codex added the banned list in 2001, the substances that formed the acceptable list were not agreed until 2011, and then with the proviso that they were to be further considered reviewed against previously agreed criteria.

An eWG was set up at the last meeting of CCFO in 2013, and the physical meeting of that group took place before the opening of the plenary session in Melaka. Following introductions, the EU delegate proposed that the EU list of acceptable previous cargoes should form the majority basis for the Codex list. This proposal was to be expected as the EU had spent a substantial amount of money commissioning the European Food Safety Authority (EFSA) to examine all the previous cargoes that were on the Codex original list. However, the proposal was not accepted by the members of the eWG, who proceeded to discuss each individual material or group of materials on the list. Due to the small quantities of some substances that are transported internationally, there was much discussion on the minimum volume that should be considered as bulk. The eWG members finally agreed that 25 metric tons should be considered as the minimum quantity, equivalent to the content of a road tanker.

The subject of flavoring agents was raised by the US delegation. The United States previously had not accepted their presence on the acceptable list because, although flavors have been evaluated by the Joint FAO/WHO Expert Commit-

tee on Food Additives (JECFA), that evaluation was for their use as ingredients rather than as potential residues in bulk oil cargoes. However, at this eWG meeting, the US delegation proposed that flavors should be moved to the acceptable list if they have been approved as flavoring compounds in foods. Similarly, the fatty acids from naturally occurring oils and fats were also considered by the Committee to be suitable for addition to the acceptable previous cargo list.

There was also some discussion on proposals by various interested parties. For example, FOSFA (Federation of Oils, Seeds and Fats Associations Ltd.; London, UK) proposed the inclusion within the acceptable list of ethyl tertiary butyl ether, which is also shipped in large quantities as the replacement for MTBE (methyl tertiary butyl ether) in fuels. On the other hand, Canada had concerns about the acceptability of all grades of white mineral oils. In summary, the physical working group confirmed many of the substances on the acceptable list, leaving just a few for further discussions or consideration by the plenary meeting.

At the full meeting, there was further general discussion on previous cargoes and it was finally agreed that 94 substances could be considered as acceptable. There was also discussion on the few extra items mentioned above, and eventually 23 substances were agreed and prioritized to be put forward to JECFA for its opinion. The committee also agreed on a procedure that would allow substances to be added or removed from the list in the light of further data that may become available. See <http://tinyurl.com/CCFO-report> for the full report.

The second eWG that met before the plenary session discussed progress that had been made on the development of the Proposed Draft Standard for Fish Oils. At the plenary meeting, the Delegation of Switzerland, which chaired this eWG, reported that agreement had been reached on many parts of the standard. Even so, the CCFO considered the revised standard on a section-by-section basis. These considerations included discussions on named fish oils and their characteristic data, the required quality parameters, the allowed food additives, and any possible contaminants. Discussion on these aspects was influenced by the national interests of the various producers and the need to include local varieties. Nevertheless, progress was made and it was agreed to forward the proposed draft standard to the Codex Alimentarius Commission for adoption at Stage 5.

Several proposals presented at the CCFO meeting concerned the amendment of the Standard for Named Vegetable Oils. These included revision of the limits of oleic and linoleic acids for sunflowerseed oil, the addition of high-oleic soybean oil, the addition of palm oil with high oleic acid, and the fatty acid composition and other quality factors in peanut (groundnut) oil. In addition, a paper on cold-pressed oils was presented by Iran. The problem with most of these proposed changes is that the volume of the oils traded is rather small and Codex has rules concerning changes that are allowed to the standards. There is sympathy for the producers wanting to get their own traits recognised within Codex but there is reluctance to change the limits within

the standards for small variations that are often not traded on a worldwide basis. This was the reason that the United States withdrew its proposal on high-oleic soybean oil. The outcome of this session was that new work was approved for the addition of high-oleic palm oil and revision of the fatty acid composition of peanut oil.

In a similar vein, there have been many discussions within CCFO on the properties of non-Mediterranean-basin olive oil. These discussions have been dominated by the EU and the International Olive Council and their member countries. There is no doubt that the EU is the most important producer and consumer of olives and olive oil, and it has vigorously specified the chemical and organoleptic properties of olive oil to prevent fraud. However, these specifications have prevented the minor producers such as Argentina, Australia, and the United States from selling their “nonstandard” olive oil as olive oil.

As in other years, there were several negotiating meetings held outside the main session, and yet again the EU maintained its position. Following a difficult round of word-smithing, which later seemed far removed from the real world of selling fresh, good-quality olive oil to the consumer, the scope of the new work was agreed, and a new eWG will be chaired by Argentina and co-chaired by Australia and Italy. Although reconciliation seems unlikely, at least the major participants will be exchanging views on possible solutions.

Almost the final item on the agenda was a proposal from FOSFA to revise the Code of Practice so that it more closely matches the international trade. Currently, the trade need not consider substances that are not on either the acceptable or banned lists as it trades on either acceptable list terms or banned list terms. The Codex Code states that these medium-risk substances may be used only if they are approved by the importing country. Unfortunately, the FOSFA proposal did not receive enough support from members to be further investigated, but, fortunately, nobody trades on Codex Code terms. This may cause problems to international trade if companies or countries do decide to use the current Codex Code as the basis of their trades or regulations.

To summarize, progress was made during this CCFO meeting on several fronts that could benefit the industry. The standard for fish oils was advanced to the next stage. The few contentious substances in the acceptable list were prioritized and submitted to JECFA, which may in turn lead to an internationally agreed list, a simplification that would help the shipping of oils and fats. A few options that would enable smaller producers to benefit from particular plant traits were added to the project list and, finally, discussions on solving the olive oil specification conundrum were revived.

The next CCFO meeting will be held in Malaysia in 2017.

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# Cacao: sustainability and flavor preservation

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

**Leslie Kleiner**

The flavor of chocolate evokes warm feelings. When I think of chocolate, I remember the traditional *chocolate con churros* we devoured as kids to celebrate the Argentine Independence Day. Others may recall the chocolate milk, chocolate bars, or succulent truffles of their youth. Consequently, as world population growth further increases the demand for cacao, there is concern not only about sustaining cocoa production but also about maintaining the one very specific trait of cacao that makes it so memorable: its flavor.

In its 2014 report, the International Cocoa Organization estimated that the 2012/2013 season experienced a cocoa supply deficit of about 207,000 metric tons (MT). The estimated cocoa production for that season was 3.928 million MT, with the Americas producing 622,000 MT, and Africa, Asia, and Oceania producing the difference. To learn how such deficits might impact the flavor of chocolate, I interviewed experts from Seguíne Cacao (a consultant to Guittard Chocolate), the US Department of Agriculture (USDA)—Agricultural Research Service (ARS), Barry Callebaut LLC, The Hershey Company, and Nahua Chocolate. We discussed the differences in flavor between Latin American cacao and that grown in other regions, threats to flavor preservation in Latin America, strategies to ameliorate these threats, and the impact chocolate making has on flavor.

## A flavorful journey through time and space

Ed Seguíne (founder of Seguíne Cacao, consultant to Guittard Chocolate, and former leader of the Mars Cocoa Sensory

Science Program at Mars Inc.) explained that it is difficult to understand the geography of cocoa flavors or potential threats to cacao production without knowing some agricultural history. This is because the many different cacao groups that exist today were originally classified based on their morphological traits and geographical origin.

Note that cacao (*Theobroma cacao* L.) refers to a tree of the *Theobroma* genus and Malvaceae family which was originally native to the upper and lower Amazonian rainforests. Cocoa is the dried seed (bean) which is taken from the fruit (pod) of the tree. Pre-Columbian civilizations transferred some varieties of cacao from their center of origin in the upper Amazon to Mesoamerica, where the plants flourished. Centuries later, other cacao types were transported and introduced to Africa and Indonesia. These new generations of cacao grown abroad experienced the “founder effect”—a loss of genetic variation that occurs when a colony is started from only a few members of an original population. In this case, a small genetic pool from the native Latin American cacao served as the main source of genetic traits in Africa and Indonesia, which affected the flavor traits that were expressed. Although the historical

movement of cacao across the continents is quite complex and occurred over many centuries, the modern introduction of cacao varieties (chosen for their productivity and disease resistance) has led to the gradual broadening of many flavor characteristics.

Seguine explained the importance of assessing flavor after fermentation of the bean and during other processing stages. After fermentation, Central American cocoa from El Salvador, Honduras, and Costa Rica has fruity and slightly citrus-like notes. In contrast, South American cocoa exhibits a wider diversity of flavor, with additional floral notes such as orange blossom, jasmine, and rose, as well as green and earthy notes. West African cocoa of the Amelonado group (which comes from Brazil) has fewer fine notes but has a deep rich chocolate flavor with moderate bitterness and astringency. Therefore, much of West African cocoa, along with cocoa varieties that have not retained particularly desirable flavor traits, is used as bulk and blended with finer flavors from Latin American cocoa. When considering responses to the factors that threaten cacao production, it is important to keep in mind that the flavor of cocoa is not homogenous around the world. “Cacao breeders get what they measure,” Seguine said. “If flavor is not in the measures, they risk getting cacao with less desirable flavor.”

### Threats to production, a decoded genome, and a return to flavor

Keeping the importance of flavor in mind and realizing that flavor depends on cacao type, its genetic traits, and its geographical location, it is easy to understand why threats to cocoa production worldwide can be catastrophic. Although various factors can affect cacao production, disease is the most prominent. Some of the diseases caused by fungal infections are black pod (*Phytophthora palmivora*, and *P. capsici*), witches’ broom (*Moniliophthora perniciosa*), frosty pod (*Moniliophthora rorei*), *mal de machete* or Ceratocystis wilt (*Ceratocystis cacaofunesta*) and black root rot (*Rosellinia pepo*, *R. bunodes*, and *R. paraguayensis*), in descending order of production losses in Latin America. Viral diseases are also a threat, but they are most common in other regions [1]. Although annual losses vary per region, there has been a tragic precedence of production losses in Latin America. A witches’ broom infestation in Brazil caused cacao production in that country alone to drop by about 75% over a span of just 10 years [2]. Due to the severity of disease, and the threat to both yields in production and to flavor preservation, diverse strategies are being implemented and/or studied to improve the overall production and quality of the cacao bean.

One recommended propagation method for a commercial production system is to use “hybrid seeds” from controlled-pollination in which the seeds are obtained from crossing at least two productive parental clones. However, as Ricardo Goenaga, a crop physiologist and Research Leader at the USDA--ARS Tropical Agriculture Research Station in Puerto Rico, pointed out, the success

## Further reading

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2. Hebbbar, P.K., Cacao diseases: a global perspective from an industry point of view, *Phytopathology* 97: 1658–1663, 2007.
3. Motamayor, J.C., *et al.*, Geographic and genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao* L), *PLoS ONE* 3:e3311, 2008.
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rate of this technique to produce high yields remains to be proven in long-term experiments. Other techniques for cacao propagation include the use of rooted cuttings or grafting, but these methods are time consuming and/or dependent upon cacao genotype compatibilities.

Somatic embryogenesis using tissue culture techniques is an area growing in popularity as a method to propagate cacao. It is worth noting that until recently, the classification of cacao was based on morphology and geographical region. However, in a joint collaborative effort by USDA--ARS and Mars Inc., scientists used molecular technology to classify more than 1,000 cacao accessions and found 10 major groups: Amelonado, Criollo, Nacional, and others [3]. This cacao classification differs from the previous morpho-geographical classification and is a more accurate description of the genetic diversity in cacao. Consequently, by understanding genetic diversity and differences among cacao clones it is then possible to develop disease-resistant cultivars and subsequently increase yield. However, this begs the question: Is it possible to increase disease resistance and yield, while also preserving flavor?

When asked this question, Ed Seguine quickly mentioned the success story developed by *Centro Agronómico Tropical de Investigación y Enseñanza* (CATIE), the Tropical Agronomy Center for Research and Education, in Costa Rica). CATIE had not only included disease-resistance traits in its breeding program, but also traits on flavor. The seeds that resulted had significant yield potential, high resistance to frosty pod and black pod, as well as desirable fruity notes. Two of these CATIE disease-resistant beans participated in the 2009 International Cocoa Awards at the *Salon du Chocolat* in Paris, and both received awards for their exquisite flavor notes.

This outcome is very important because the most ubiquitous cacao clone identified on Latin American plantations, *Colección Castro Naranjal-51* (CCN-51), has an undesirable flavor profile when fermented using standard Ecuadorian protocols. The flavor of the ubiquitous clone, which is known for its bitterness, astringency, and acidity, can be greatly improved with alternative fermentation protocols. Unfortunately, such protocols are problematic for other locally grown clones, which consequently cannot be planted with CCN-51. Although the CCN-51 clone

has been used as an optimal parent in many breeding programs due to its high yield and resistance to diseases such as witches' broom, its undesirable flavor profile makes it a low quality bean. Therefore, using this inferior bean to dilute better quality beans (as occurred in the reported adulteration of the Nacional cacao variety from Ecuador) presents a concern to Ecuadorian exporters and cacao importers worldwide [3].

The increasing demand for fine flavors has prompted efforts to distinguish growers of the CCN-51 variety from those growing finer aromatic beans. Furthermore, cacao beans such as those produced by CATIE can also aid in crop yield, flavor preservation, and the development of complex sensory notes.

### Flavor in chocolate making and coatings

Until now, we have discussed factors that affect cacao production, crop yield, and flavor conservation of the bean (seed). However, much of the chocolate flavor develops during the chocolate-making process. To understand these processes and their role in flavor development, I interviewed Raymond Major (senior manager, sustainability initiatives, The Hershey Company). Major explained how in fine chocolates, such as Hershey's Scharffen Berger brand, cacao beans are carefully blended for an optimal flavor balance. The fine flavors of exceptionally flavorful beans, such as those from Ecuador, Peru, and the Dominican Republic, must be carefully preserved and/or enhanced in later processes. Since all Latin American beans sourced for Scharffen Berger products are certified by an independent certification that validates environmental, ecological, social, and economic standards, it is crucial to preserve the high quality of the bean throughout all processes.

Major described how the initial steps consist of fermentation and drying of the beans by the bean producers. Fermentation refers to a key step in which the pulp (not the exposed seed) is fermented to enhance or modify the flavor notes that are present in the bean. After these steps are completed and the beans are sourced to the factory, they are roasted at temperatures that are mild enough to retain volatile aromatics while developing the desirable chocolate flavor. The beans are then cooled, broken, and winnowed to remove the shells and obtain the remaining edible nibs. The nibs are ground using a *melangeur* (granite roller) to form a paste called "chocolate liquor." This process differs from a more common industrial process that uses pin mills in lieu of a *melangeur*, but it is the delicate motions of the *melangeur* that preserves the delicate fruity and winery notes of the beans. The liquor, mixed with cane sugar and Bourbon vanilla, is then placed in a refiner-conch, where it is mixed for a whole day to further develop the flavors before molding into bars.

Daniela Vásquez Villegas (head chocolatier, Nahua Chocolate, Costa Rica) agrees that flavor development in chocolate depends on several factors, including the cacao

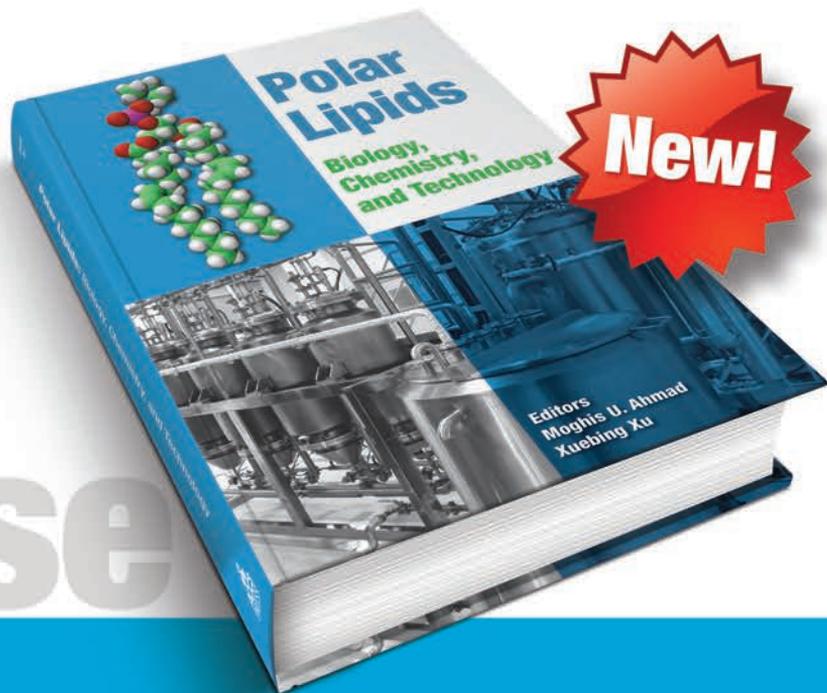
cultivar, fermentation, roasting, and subsequent chocolate making steps. Vásquez Villegas explained that Nahua Chocolates uses 100% Costa Rican cacao sourced from small producers in the Upala region, a region known for high-quality cacao beans. Furthermore, to help increase yield and productivity and ensure a reliable cacao source, Nahua Chocolate offers technical assistance to farmers. The assistance not only results in a better quality bean, but also increases the sense of pride within the agricultural and chocolate-producing community. The quality of the Upalan bean, which is characterized by strong cacao notes with additional caramel and dried fruit notes, is preserved by a careful process that requires attention to detail and patience. In contrast with other industrial processes for chocolate making, Nahua roasts the beans at a lower temperature but for a much longer period of time than most makers do. The delicate process develops and enhances flavor notes present in the beans, so it needs to be carefully monitored from beginning to end.

Daniel Kazmierczak (director of applications and technical services, compounds and fillings, Barry Callebaut LLC) also emphasized the importance of cocoa bean origins and processing conditions for flavor development in chocolate coatings. However, Kazmierczak explained that coatings (also called "compound coatings") have different applications than chocolate. Therefore, coatings are produced using different ingredients and processes. For example, coatings can be formulated using various vegetable oils and flavors, and their melting properties can be targeted to specific melting curves needed to make various confections. Cocoa powder is the main ingredient for flavor development in chocolate coatings, but additional flavor notes such as milk and/or cream, may also be used. Since coatings lack a true conching phase, flavors are developed at different stages than those seen during chocolate making.

As demand for cacao grows, so do joint efforts between private and public sectors to protect its supply and flavors. Preserving the flavor of chocolate is crucial to all cultures, and not just in Latin America. As Ed Seguire said, "Chocolate is like the music that plays in a symphony hall; even when the instruments are put away, the memory lingers with us."

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# The (*n*-6) Family of Polyunsaturated Fatty Acids

*Lipid Snippets is a regular Inform column that features select content from The AOCS Lipid Library (<http://lipidlibrary.aocs.org/>).*

The lipids of all higher organisms contain appreciable quantities of polyunsaturated fatty acids (PUFA) with methylene-interrupted double bonds, i.e. with two or more double bonds of the *cis*-configuration separated by a single methylene group. The term ‘homallylic’ is occasionally used to describe this molecular feature.

In higher plants, the number of double bonds in fatty acids only rarely exceeds three, but in algae and animals there can be up to six. Two principal families of polyunsaturated fatty acids occur in nature that are derived biosynthetically from **linoleic** (9-*cis*,12-*cis*-octadecadienoic) and  **$\alpha$ -linolenic** (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic) acids (Fig. 1).

In the shorthand nomenclature, these are designated 9*c*,12*c*-18:2 and 9*c*,12*c*,15*c*-18:3 respectively. The number before the colon specifies the number of carbon atoms, and that after the colon, the number of double bonds. The position of the terminal double bond can be denoted in the form (*n*-*x*), where *n* is the chain-length of the fatty acid and *x* is the number of carbon atoms from the last double bond, assuming that all the other double bonds are methylene-interrupted. Thus linoleate and  $\alpha$ -linolenate are 18:2(*n*-6) and 18:3(*n*-3), respectively (18:2 $\omega$ 6 and 18:3 $\omega$ 3 in the older literature).

Both of the parent fatty acids can be synthesized in plants, but not in animal tissues, and they are therefore essential dietary components. Polyunsaturated fatty acids can be found in most lipid classes, but they are especially important as constituents of the phospholipids, where they appear to confer distinctive properties to the membranes, in particular by decreasing their rigidity. The exception is the sphingolipids, where they are rarely detected in other than trace amounts.

## The (*n*-6) family

**Linoleic acid** is a ubiquitous component of plant lipids, and of all the seed oils of commercial importance. For example, corn, sunflower, and soybean oils usually contain over 50% of linoleate, and safflower oil contains up to 75%. Although all the linoleate in animal tissues must be acquired from the diet, it is usually the most abundant dienoic fatty acid in mammals (and in most lipid classes) typically at levels of 15 to 25%, although it can amount to as much as 75% of the total fatty acids of heart cardiolipin. It is also a significant component of fish oils, although fatty acids of the (*n*-3) family tend to predominate in this instance.

Analogues of linoleic acid with *trans*-double bonds are occasionally found in seed oils. For example, 9*c*,12*t*-18:2 is reported from *Dimorphotheca* and *Crepis* species, and 9*t*,12*t*-18:2 is found in *Chilopsis linearis*.

The remaining members of the (*n*-6) family of fatty acids are synthesized from linoleate in animal and plant tissues by a sequence of elongation and desaturation reactions as described below. The intermediates can function as essential fatty acids also. Shorter-chain components may be produced by *alpha* or *beta*-oxidation.

**$\gamma$ -Linolenic acid** (‘GLA’ or 6-*cis*,9-*cis*,12-*cis*-octadecatrienoic acid or 18:3(*n*-6)) is usually a minor component of animal tissues in quantitative terms (< 1%), as it is rapidly converted to higher metabolites. It is found in a few seed oils, and those of

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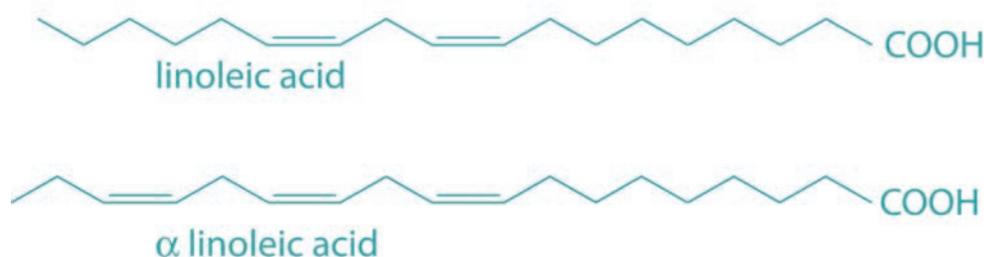


FIG. 1. Linoleic and  $\alpha$ -linolenic acids



FIG. 2. Arachidonic acid

evening primrose, borage and blackcurrant have some commercial importance. Evening primrose oil contains about 10% GLA, and is widely used both as a nutraceutical and a medical/veterinary product.

11-*cis*,14-*cis*-Eicosadienoic acid (20:2(*n*-6)) is a common minor component of animal tissues. 8-*cis*,11-*cis*,14-*cis*-Eicosatrienoic acid (dihomo- $\gamma$ -linolenic acid or 20:3(*n*-6)) is the immediate precursor of arachidonic acid, and of a family of eicosanoids (PG<sub>1</sub> prostaglandins). However, it does not accumulate to a significant extent in animal tissue lipids, and is typically about 1-2% of the phospholipid fatty acids.

**Arachidonic acid** (5-*cis*,8-*cis*,11-*cis*,14-*cis*-eicosatetraenoic acid or 20:4(*n*-6)) is the most important metabolite of linoleic acid in animal tissues, both in quantitative and biological terms. It is often the most abundant polyunsaturated

component of the phospholipids, and can comprise as much as 40% of the fatty acids of phosphatidylinositol. As such, it has an obvious role in regulating the physical properties of membranes, but the free acid is also involved in the mechanism by which apoptosis is regulated (Fig 2).

Meat is the main dietary source in humans. While arachidonate is present in all fish oils, polyunsaturated fatty acids of the (*n*-3) families tend to be present in much larger amounts. Arachidonic acid is frequently found as a constituent of mosses, liverworts, and ferns, but there appears to be only one definitive report of its occurrence in a higher plant (*Agathis robusta*). The fungus *Mortierella alpina* is a commercial source or arachidonate via a fermentation process.

Several families of **eicosanoids** are derived from arachidonate, including prostaglandins (PG<sub>2</sub> series), thromboxanes, leukotrienes, and lipoxins, with **phosphatidylinositol** being the primary source. These have an enormous range of essential biological functions that are discussed in elsewhere in these web pages. In addition, **2-arachidonoylglycerol** and anandamide **N-arachidonylethanolamine** have important biological properties as endocannabinoids, although they are minor lipids in tissues in quantitative terms.

**4,7,10,13,16-Docosapentaenoic acid** (22:5(*n*-6)) is usually a relatively minor component of animal lipids, but it is the main C<sub>22</sub> polyunsaturated fatty acid in the phospholipids of testes. It can amount to 70% of the lysobisphosphatidic acid in this tissue, for example. In this instance, C<sub>22</sub> fatty acids of the (*n*-3) family are present at relatively low levels, in contrast to most other reproductive tissues.

**Other** fatty acids of the (*n*-6) family that are found in animal tissues include 22:3(*n*-6) and 22:4(*n*-6). The last of these, 7,10,13,16-docosatetraenoic or adrenic acid, is a significant component of the phospholipids of the adrenal glands and of testes. Tetra- and pentaenoic fatty acids of the (*n*-6) family from C<sub>24</sub> to C<sub>30</sub> have been found in testes, and even longer homologues occur in retina. Those in testes are known to be essential for male fertility and sperm maturation. Very-long-chain fatty acids of this type were first reported from human brain in patients with the rare inherited disorder, Zellweger's syndrome, but it is now established that such fatty acids with up to 38 carbon atoms and with from 3 to 6 methylene-interrupted double bonds are present at low levels the brain of normal young humans, with 34:4(*n*-6) and 34:5(*n*-6) tending to predominate. The function of these is not known.

The most highly unsaturated fatty acid of the (*n*-6) family to have been characterized are 28:7(*n*-6) (4,7,10,13,16,19,22-octacosaeptaenoate), which has been found in the lipids of marine dinoflagellates and herring muscle, and 4,7,10,13,16,19,22,25,28-tetracontanonaenoic acid (34:9(*n*-6)) from the freshwater crustacean species *Bathynella natans*. ■

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## Call for Nominations

# Stephen S. Chang Award

### The Award

The Stephen S. Chang Award recognizes a scientist, technologist, or engineer who has made significant and distinguished accomplishments in basic research that must have been utilized by industries for the development or improvement of products related to lipids. The awardee may be recognized for either one major breakthrough or an accumulation of publications.

A prospective recipient must agree to be present for acceptance of the award and to deliver an award address at the 107th AOCs Annual Meeting & Expo. The award is made without regard for national origin, place of residence, race, color, creed, or gender.



The Stephen S. Chang Award recognition shall consist of an honorarium and a jade galloping horse symbolizing the award. The late Stephen S. Chang, an AOCs past president, and his wife, Lucy D. Chang, sponsor the award.

### Nomination Procedures

*Nominations for the 2016 award must be submitted before October 15, 2015.*

The suggestions listed below may be helpful to nominators in addressing the mandatory criteria of industrial utilization.

1. Documentation of the application of research
  - a. Patents received, licensing arrangements
  - b. Specific examples of industrial use
2. Documentation for the development or improvement of products related to lipids
  - a. Listing of new products, manufacturers, sales history
  - b. Manufacturers' testimonials regarding product improvement resulting from their direct utilization of the basic research in specific products with comparative figures on sales or consumer acceptance

The nomination must include a letter from the nominator, at least three supporting letters, the nominee's *curriculum vitae*, and a list of major relevant publications, including patents.



# PATENTS

## Methods for joint lubrication and cartilage wear prevention making use of glycerophospholipids

Halperin, G., *et al.*, Hadasit Medical Research Services and Development Ltd., Technion Research and Development Foundation Ltd., and Yissum Research Development Co. of Hebrew University Jerusalem, US8895054, November 25, 2014

The present invention concerns methods of joint lubrication and/or prevention of cartilage wear making use of liposomes having membranes with at least one phospholipid (PL) of the group consisting of a glycerophospholipid (GPL) having two, being the same or different, C12-C16 hydrocarbon chain and a sphingolipid (SPL) having a C12-C18 hydrocarbon chain, the one or more membranes having a phase transition temperature in which solid ordered (SO) to liquid disordered (LD) phase transition occurs, the phase transition temperature being within a temperature of about 20 °C to about 39 °C for lubrication of joints.

## Agglomerated oil impregnated psyllium husk

Jacobsen, G. and H.T. Hesselballe, Biofiber Damino A/S, US8895085, November 25, 2014

There is provided agglomerated oil impregnated psyllium husk and to a method for manufacturing the agglomerated oil impregnated psyllium husk. Specifically the present invention provides compositions comprising agglomerates of psyllium husks, wherein the husks have been subjected to treatment with an oily component (e.g. vegetable oil, such as rapeseed oil) prior to a drying process, such as a fluidized bed process, wherein the husks are agglomerated with a saccharide containing aqueous suspension/solution sprayed onto the husks.

## ZnO nanoparticle catalysts for use in biodiesel production and method of making

Yan, S., *et al.*, US. Department of Energy and Wayne State University, US8895764, November 25, 2014

A method of forming a biodiesel product and a heterogeneous catalyst system used to form said product that has a high tolerance for the presence of water and free fatty acids (FFA) in the oil feedstock is disclosed. This catalyst system may simultaneously catalyze both the esterification of FFA and the transesterification of triglycerides present in the oil feedstock. The catalyst system is comprised of a mixture of zinc oxide and a second metal oxide. The zinc oxide includes a mixture of

amorphous zinc oxide and zinc oxide nanocrystals, the zinc nanocrystals having a mean grain size between about 20 and 80 nm with at least one of the nanocrystals including a mesopore having a diameter of about 5 to 15 nm. Preferably, the second metal oxide is a lanthanum oxide, the lanthanum oxide being selected as one from the group of  $\text{La}_2\text{CO}_3$ ,  $\text{LaOOH}$ , and combinations or mixtures thereof.

## Biodiesel cold flow improver

Scanlon, E., *et al.*, BASF Corp. and Ciba Corp., US8900333, December 2, 2014

The present invention is directed to the use of alkyl(meth)acrylate polymers or copolymers of the formula (I) In-Poly-(E) $\gamma$  (I) as cold flow improvers in biodiesel fuel (or bio-fuel) and biodiesel compositions incorporating said polymers or copolymers, obtained by nitroxyl mediated controlled free radical polymerization, wherein In is the initiator fragment starting the polymerization reaction; E is an end group bearing at least one stable free nitroxyl radical, which is bound via an oxygen atom to the polymer or copolymer; or a group which results from a substitution or elimination reaction of the attached stable free nitroxyl radical; Poly is any polymer or copolymer formed from ethylenically unsaturated monomer(s); and  $\gamma$  is a number 1 or greater than 1 indicating the average number of end groups E attached to Poly.

## Multi-functional wood preservatives based on a borate/fatty acid combination

Coleman R.D., Summerdale Inc., US8900720, December 2, 2014

Wood preservatives, methods for protecting wood and wood-based products and/or structures utilizing the preservatives, and treated wood and wood-based articles and/or structures incorporating the preservatives are described. Methods are also provided for remediating structures already infected with mold. The subject wood preservative formulation includes a borate compound and a fatty acid. The addition of an emulsifier to the formulation further facilitates application of the treatment. The combination of a borate compound and fatty acid combination typically provide a synergistic effect compared to the additive result provided by the combination's individual components.

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



# EXTRACTS & DISTILLATES

## Long-chain omega-3 from low-trophic-level fish provides value to farmed seafood

Bibus, D.M., *Lipid Technol.* 27: 55–58, 2015  
<http://dx.doi.org/10.1002/lite.201500006>, open access.

Low-trophic-level fish are a crucial source of long-chain (LC) omega-3 fatty acids for farmed fish and humans. Many farm-raised fish species have a clear need for these nutrients. Farmed fish deposit the LC omega-3s in their flesh and transfer them up the food chain. However, the content of LC omega-3s in farm-raised seafood continues to decline, while the content of shorter-chain plant-sourced omega-3s, and pro-inflammatory omega-6s continue to increase. This reduces its nutritional worth. The value of low-trophic-level fish is often viewed merely as its price at the dock. Some reports and metrics steer public attention towards the mass balance between quantities of low-trophic-level fish and farmed seafood. However, the nutritional value of seafood is more important than its mere quantities. The role of low-trophic-level fish in human nutrition, health, and wellbeing is a fundamental component of its economic value to society.

## Docosahexaenoic acid and traumatic brain injury

Thau-Zuchman, O., *Lipid Technol.* 27: 63–66, 2015  
<http://dx.doi.org/10.1002/lite.201500008>.

Traumatic brain injury (TBI) is becoming increasingly recognized as a major cause of disability, with a significant impact on health costs both in the developed and developing world. At present, there is no effective treatment for this type of acute neurological injury. Docosahexaenoic acid (DHA) is an abundant polyunsaturated fatty acid in the brain, known to have a significant structural role as well as functional roles. Preclinical accumulating evidence indicates that DHA has potential as a restorative therapeutic agent for traumatic brain injury.

## Lipids in cheese

Tunick, M.H., *Lipid Technol.* 27: 83–85, 2015  
<http://dx.doi.org/10.1002/lite.201500015>.

Lipids are present in cheese at levels above 20% and are analyzed by several techniques. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) are used to examine the microstructure, gas chromatography is employed to look at fatty acid composition, and differential

scanning calorimetry is utilized to examine the melting profile. Differences resulting from storage, organic feeding regimen, substitution of milk from a different species, and homogenization may be determined.

## Circadian regulators of intestinal lipid absorption

Hussain, M.M. and X. Pan, *J. Lipid Res.* 56: 761–770, 2015  
<http://dx.doi.org/10.1194/jlr.R051573>.

Among all the metabolites present in the plasma, lipids, mainly triacylglycerol and diacylglycerol, show extensive circadian rhythms. These lipids are transported in the plasma as part of lipoproteins. Lipoproteins are synthesized primarily in the liver and intestine and their production exhibits circadian rhythmicity. Studies have shown that various proteins involved in lipid absorption and lipoprotein biosynthesis show circadian expression. Further, intestinal epithelial cells express circadian clock genes and these genes might control circadian expression of different proteins involved in intestinal lipid absorption. Intestinal circadian clock genes are synchronized by signals emanating from the suprachiasmatic nuclei that constitute a master clock and from signals coming from other environmental factors, such as food availability. Disruptions in central clock, as happens due to disruptions in the sleep/wake cycle, affect intestinal function. Similarly, irregularities in temporal food intake affect intestinal function. These changes predispose individuals to various metabolic disorders, such as metabolic syndrome, obesity, diabetes, and atherosclerosis. Here, we summarize how circadian rhythms regulate microsomal triglyceride transfer protein, apoAIV, and nocturnin to affect diurnal regulation of lipid absorption.

## Consumption of fatty foods and incident type 2 diabetes in populations from eight European countries

Buijsse, B., *et al.*, *Eur. J. Clin. Nutr.* 69: 455–461, 2015  
<http://dx.doi.org/10.1038/ejcn.2014.249>.

Diets high in saturated and trans fat and low in unsaturated fat may increase type 2 diabetes (T2D) risk, but studies on foods high in fat per unit weight are sparse. We assessed whether the intake of vegetable oil, butter, margarine, nuts, and seeds and cakes and cookies is related to incident T2D. A case-cohort study was conducted, nested within eight countries of the European Prospective Investigation into Cancer (EPIC), with 12,403 incident T2D cases and a subcohort of 16,835 people, identified from a cohort of 340,234 people. Diet was assessed at baseline (1991–1999) by country-specific questionnaires. Country-specific hazard ratios (HRs) across four categories of fatty foods (nonconsumers and tertiles among consumers) were combined with random-effects meta-analysis. After adjustment not including body mass index (BMI), nonconsumers of butter, nuts and seeds and cakes

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and cookies were at higher T2D risk compared with the middle tertile of consumption. Among consumers, cakes and cookies were inversely related to T2D (HRs across increasing tertiles 1.14, 1.00 and 0.92, respectively;  $P$ -trend  $<0.0001$ ). All these associations attenuated upon adjustment for BMI, except the higher risk of nonconsumers of cakes and cookies (HR 1.57). Higher consumption of margarine became positively associated after BMI adjustment (HRs across increasing consumption tertiles: 0.93, 1.00 and 1.12;  $P$ -trend 0.03). Within consumers, vegetable oil, butter and nuts and seeds were unrelated to T2D. Fatty foods were generally not associated with T2D, apart from weak positive association for margarine. The higher risk among nonconsumers of cakes and cookies needs further explanation.

## Effect of processing of cow milk by high pressures under conditions up to 900 MPa on the composition of neutral, polar lipids and fatty acids

Rodríguez-Alcalá, L.M., *et al.*, *Food Sci. Techn.* 62: 265–270, 2015, <http://dx.doi.org/10.1016/j.lwt.2014.12.052>.

Thermal processing of milk promotes oxidation reactions and changes in organoleptic characteristics. Application of High Pressure Processing (HPP) is a promising alternative but little is known about the impact on milk compounds, mainly lipids. This research aims to study the possible alteration of the composition of milk lipids and fatty acids as result of HPP using 250–900 MPa. Thus, two cow milk batches were assayed and the composition in tri-, di-, monoacylglycerols, cholesterol, cholesterol esters, free fatty acids, phospholipids and fatty acids analyzed. It was found differences ( $p < 0.05$ ) among batches in the distribution of triacylglycerols (CN34, CN50 and CN54) and also in the concentration of diacylglycerols, monoacylglycerols, cholesterol, phosphatidylethanolamine and phosphatidylinositol and unsaturated fatty acids (C18:1 10t, C18:1 9c, C18:1 11c, C18:1 16t+14c, C18:2 9c,12c, C18:2 9c,11t and C20:1 9c). Data from the current study showed that processing by HPP up to 900 MPa did not produce significant changes to the lipid classes or fatty acid composition of milk fat.

## Changes in lipids and volatile compounds of oat flours and extrudates during processing and storage

Lampi, A.-M., *et al.*, *J. Cereal Sci.* 62: 102–109, 2015 <http://dx.doi.org/10.1016/j.jcs.2014.12.011>.

Oats is valuable raw material, but it needs to be heat-treated to inactivate lipid degrading enzymes that would deteriorate its sensory quality. The aim was to study if extrusion could replace



## Journal of Surfactants and Detergents (May)

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# Lipids

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- da Costa F., R. Robert, C. Quéré, G.H. Wikfors, and P. Soudant, Essential fatty acid assimilation and synthesis in larvae of the Bivalve *Crassostrea gigas*
- Tareq, F.S., H.-S. Lee, Y.-J. Lee, J.S. Lee, and H.J. Shin H.J., Ieodoglucomide C and ieodoglycolipid, new glycolipids from a marine-derived bacterium *Bacillus licheniformis* 09IDYM23

traditional heat treatment in production of whole meal oats with stable lipids. Flours from non-heat-treated (NHT) oat grains were extruded under four conditions and subjected to 15-week storage. Stability of the extrudates as well as the NHT and heat-treated oat grains were studied for hydrolytic and oxidative reactions by measuring neutral lipid profiles and volatile products. In the NHT oat grains, lipid hydrolysis started immediately after milling, which also promoted lipid oxidation during storage. Enzymatic degradation of lipids could effectively be prevented by extrusion even at the lowest temperature of 70 °C. The extrusion temperature could be increased to 110 °C without subjecting the lipids to non-enzymatic oxidation. However, by increasing the temperature to 130 °C, lipid oxidation was promoted, which also resulted in losses of neutral lipids over time. Hexanoic acid became the major volatile product in oat extrudates during extensive lipid oxidation instead of hexanal, which is a commonly used lipid oxidation indicator. In conclusion, lipids in oat grains could be stabilized by extrusion even at a temperature of 70 °C.

## Phytosterols and their extraction from various plant matrices using supercritical carbon dioxide: a review

Uddin, M.D., *et al.*, *J. Sci. Food Agric.* 95: 1385–1394, 2015  
<http://dx.doi.org/10.1002/jsfa.6833>.

Phytosterols provide important health benefits: in particular, the lowering of cholesterol. From environmental and commercial points of view, the most appropriate technique has been searched for extracting phytosterols from plant matrices. As a green technology, supercritical fluid extraction (SFE) using carbon dioxide (CO<sub>2</sub>) is widely used to extract bioactive compounds from different plant matrices. Several studies have been performed to extract phytosterols using supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) and this technology has clearly offered potential advantages over conventional extraction methods. However, the efficiency of SFE technology fully relies on the processing parameters, chemistry of interest compounds, nature of the plant matrices and expertise of handling. This review covers SFE technology with particular reference to phytosterol extraction using SC-CO<sub>2</sub>. Moreover, the chemistry of phytosterols, properties of supercritical fluids (SFs) and the applied experimental designs have been discussed for better understanding of phytosterol solubility in SC-CO<sub>2</sub>.

## Developing an integrated supercritical fluid biorefinery for the processing of grains

Temelli, F., *et al.*, *J. Supercrit. Fluids* 96: 77–85, 2015  
<http://dx.doi.org/10.1016/j.supflu.2014.09.028>.

With the rapid growth of the bioeconomy, there are major developments in the biorefinery concept to maximize utilization

of renewable resources, including grains of cereals and oilseeds, targeting applications not only in food, nutraceutical, pharmaceutical and cosmetic products but also in biofuel, biochemical and biomaterial sectors. Supercritical carbon dioxide (SC-CO<sub>2</sub>) technology could have a major role to play in modern biorefineries. Know-how has been building on individual unit operations based on SC-CO<sub>2</sub>, especially extraction, fractionation and reactions involving lipids and particle formation for delivery of bioactives as summarized in this study mostly based on the previous work of the authors. Based on a solid understanding of fundamentals, the focus is now on optimal integration of these unit operations to build supercritical biorefineries. Such approaches should also consider integration of supercritical operations with conventional technologies for efficient and optimal process design. Even though the future of supercritical biorefineries seems to be bright, offering numerous advantages, there are also some substantial technical and economic challenges requiring further research.

## Extraction of carotenoids and lipids from algae by supercritical CO<sub>2</sub> and subcritical dimethyl ether

Goto, M., *et al.*, *J. Supercrit. Fluids* 96: 245–251, 2015  
<http://dx.doi.org/10.1016/j.supflu.2014.10.003>.

Algae contain lipids and functional compounds such as carotenoids. Especially, microalgae are recently focused as a source of biofuel. To extract these components, organic solvent or supercritical carbon dioxide have been used. We have been developing wet extraction process using liquefied (subcritical) dimethyl ether (DME) as solvent at around 0.59 MPa. The extraction process usually requires energy consuming drying and grinding process as in the case of supercritical CO<sub>2</sub>. We have applied liquefied DME for the extraction of lipids and functional compounds from various kinds of algae. Since the liquefied DME extraction process can eliminate the process for drying, cell disruption, and solvent evaporation, it can realize simpler and low energy consumption system. In this paper, our studies on the extraction from algae by using supercritical CO<sub>2</sub> or subcritical DME are reviewed.

## An organic solvents free bio-lipids extraction process using non-woven fabric from pretreated fermentation broth

Shang, S., *et al.*, *Chem. Eng. J.* 270: 223–228, 2015  
<http://dx.doi.org/10.1016/j.cej.2015.02.038>.

In this study, to avoid solvent contamination and decrease the cost of bio-lipids extraction, a novel non-woven fabric (NWF) was synthesized to separate bio-lipids from pretreated *Rhodotorula glutinis* fermentation broth. After fermentation with the wastewater mixture, cells were concentrated by micro-filtration and the concentrated cells were disrupted via high

pressure homogenization. Bio-lipids was extracted and separated from cell disruption suspension via NWF. The NWF can be used to carry out 25 cycles of oil extraction, which showed it was effective and easily recyclable in bio-oil recovery. The extraction temperature was also optimized, and about 25.5 mL/L of final bio-lipids product with 81.7% of the theoretical yield was achieved at 70 °C. The fatty acid compositions extracted by NMW were similar with those obtained using the conventional organic solvent extraction methods. The results showed that the novel process provided an attractive and promising way to separate bio-lipids from microorganisms.

## Algal biofuels from urban wastewaters: Maximizing biomass yield using nutrients recycled from hydrothermal processing of biomass

Selvaratnam, T., *et al.*, *Bioresour. Techn.* 182: 232–238, 2015  
<http://dx.doi.org/10.1016/j.biortech.2015.01.134>.

Recent studies have proposed algal cultivation in urban wastewaters for the dual purpose of waste treatment and bio-energy production from the resulting biomass. This study proposes an enhancement to this approach that integrates cultivation of an acidophilic strain, *Galdieria sulphuraria* 5S87.1, in a closed photobioreactor (PBR); hydrothermal liquefaction (HTL) of the wet algal biomass; and recirculation of the nutrient-rich aqueous product (AP) of HTL to the PBR to achieve higher biomass productivity than that could be achieved with raw wastewater. The premise is that recycling nutrients in the AP can maintain optimal C, N and P levels in the PBR to maximize biomass growth to increase energy returns. Growth studies on the test species validated growth on AP derived from HTL at temperatures from 180 to 300 °C. Doubling N and P concentrations over normal levels in wastewater resulted in biomass productivity gains of 20–25% while N and P removal rates also doubled.

## Mechanical extraction of oil from *Jatropha curcas* L. kernel: Effect of processing parameters

Subroto, E., *et al.*, *Ind. Crops Prod.* 63: 303–310, 2015  
<http://dx.doi.org/10.1016/j.indcrop.2014.06.018>.

Mechanical extraction is considered to be the best option for oil expression of *Jatropha curcas* in rural areas. Lab scale hydraulic pressing experiments were conducted to investigate the effect of process parameters on oil recovery from *Jatropha* kernel. The ranges of pressing parameters investigated were: compression speed, 0.05–2.5 MPa/s; applied pressure, 5–25 MPa; moisture content, 1–6%; pressing temperature, 25–105 °C; pressing time, 1–30 min; shell removal, 0–100%; preheating time, 0–30 min; and particle size, fine, coarse and

whole kernel. Chemical analyses such as acid value, phosphorus content, oxidative stability index and water content were carried out to determine the quality of the oil. Moisture content was found to influence oil recovery at any applied pressure and pressure rates. Oil recovery increased to some extent with an increase in temperature or pressing time. The preferred moisture content was found to be about 4% (w.b.). The presence of *Jatropha* shell and size reduction of the kernel reduce oil recovery. The optimum oil recovery with respect to time of pressing and oil quality was obtained when *Jatropha* kernel was pressed at 15 MPa, 90 °C, 4% (w.b.) moisture content for 10 min of pressing. The oil recovery obtained at these processing conditions was 86.1%.

## Synthesis of structured lipids by enzymatic interesterification of milkfat and soybean oil in a basket-type stirred tank reactor

Paula, A.V., *et al.*, *Ind. Eng. Chem. Res.* 54: 1731–1737, 2015  
<http://dx.doi.org/10.1021/ie503189>.

Lipase from *Rhizopus oryzae* immobilized on polysiloxane–poly(vinyl alcohol) (SiO<sub>2</sub>–PVA) was used to study the interesterification reaction of the milkfat with soybean oil in a stirred tank reactor (STR) containing baskets for the immobilized enzyme retention in two different configurations: central or lateral. The progress of the reaction was followed by determining free fatty acids, composition in triacylglycerols (TAGs), and consistency. The central basket was chosen for assessing the biocatalyst operational stability by running 10 consecutive batch assays lasting 6 h each. Non-notable deactivation of the biocatalyst was observed during the total operation time (60 h). The potential of the evaluated system to make the milkfat-based fat more spreadable under cooling temperature and with lower saturated fatty acids content was demonstrated in this study. Both evaluated basket reactor designs have shown potential to be used in interesterification reactions of industrial interest.

## Solid state fermentation by cellulolytic oleaginous fungi for direct conversion of lignocellulosic biomass into lipids: Fed-batch and repeated-batch fermentations

Cheirsilp, B. and S. Kitcha, *Ind. Crops Prod.* 66: 73–80, 2015  
<http://dx.doi.org/10.1016/j.indcrop.2014.12.035>.

Lignocellulosic wastes from palm oil mill are one of attractive feedstocks for microbial lipid production by oleaginous microorganisms because of their low cost, renewable nature



# Lipids

Since 1966

## No. 1 on the “fab 5” list

A drumroll, please: We've reached No. 1 on the “fab 5” list of pivotal original papers chosen by Editor-in-Chief Eric J. Murphy in celebration of *Lipids'* 50th volume. The No. 1 paper, a communication on the quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots, appeared in the first issue of *Lipids* in January 1966. Written by George Rouser, A.N. Siakotos, and Sidney Fleischer, the paper has been cited more than 1,385 times over the years, pointing to its importance to continuing work on the extraction and purification of lipids from biological materials. The paper is available for download at <http://tinyurl.com/Quant-Ana>.

and abundance. In this study, four filamentous fungi with cellulolytic activity were screened as potential oleaginous microorganisms for direct conversion of these lignocellulosic wastes into lipid. Among them, *Aspergillus tubingensis* TSIP9 accumulated lipid at the highest amount of  $39.5 \pm 2.2$  mg per gram dry substrate (gds), and simultaneously produced high activities of cellulase ( $2.35 \pm 0.22$  U/gds) and xylanase ( $11.83 \pm 0.18$  U/gds) through solid state fermentation (SSF) of palm empty fruit bunches (EFB). The use of EFB mixed with palm kernel cake (PK) promoted lipid production by the fungi up to  $79.9 \pm 3.5$  mg/gds. When the enzymes were extracted from the first batch and reused in the next batch, *A. tubingensis* TSIP9 produced much higher amount of enzymes and accumulated lipid faster. Fed-batch SSF with intermittent adding of EFB could be applied for lipid production but with a decrease in the enzyme activity. When repeated-batch SSF with 90% replacement with new substrate was applied, both lipid and enzymes were efficiently produced for long period of fermentation. This new strategy for solid state fermentation may contribute greatly to the commercialized enzyme and lipid productions from abundant lignocellulosic biomass.

## Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*

Qiao, K., et al., *Metab. Eng.* 29: 56–65, 2015  
<http://dx.doi.org/10.1016/j.ymben.2015.02.005>.

Conversion of carbohydrates to lipids at high yield and productivity is essential for cost-effective production of renewable biodiesel. Although some microorganisms can convert sugars to oils, conversion yields and rates are typically low due primarily

to allosteric inhibition of the lipid biosynthetic pathway by saturated fatty acids. By reverse engineering the mammalian cellular obese phenotypes, we identified the  $\Delta$ -9 stearoyl-CoA desaturase (SCD) as a rate limiting step and target for the metabolic engineering of the lipid synthesis pathway in *Yarrowia lipolytica*. Simultaneous overexpression of SCD, Acetyl-CoA carboxylase (ACC1), and Diacylglyceride acyl-transferase (DGA1) in *Y. lipolytica* yielded an engineered strain exhibiting highly desirable phenotypes of fast cell growth and lipid overproduction including high carbon to lipid conversion yield (84.7% of theoretical maximal yield), high lipid titers ( $\sim 55$  g/L), enhanced tolerance to glucose and cellulose-derived sugars. Moreover, the engineered strain featured a three-fold growth advantage over the wild type strain. As a result, a maximal lipid productivity of  $\sim 1$  g/L/h is obtained during the stationary phase. Furthermore, we showed that the engineered yeast required cytoskeleton remodeling in eliciting the obesity phenotype. Altogether, our work describes the development of a microbial catalyst with the highest reported lipid yield, titer and productivity to date. This is an important step towards the development of an efficient and cost-effective process for biodiesel production from renewable resources.

## Metabolic engineering for the production of hydrocarbon fuels

Lee, S.Y., et al., *Curr. Opin. Biotechnol.* 33: 15–22, 2015  
<http://dx.doi.org/10.1016/j.copbio.2014.09.008>.

Biofuels have been attracting increasing attention to provide a solution to the problems of climate change and our dependence on limited fossil oil. During the last decade, metabolic engineering has been performed to develop superior microorganisms for the production of so called advanced biofuels. Among the advanced biofuels, hydrocarbons possess high-energy content and superior fuel properties to other biofuels, and thus have recently been attracting much research interest. Here we review the recent advances in the microbial production of hydrocarbon fuels together with the metabolic engineering strategies employed to develop their production strains. Strategies employed for the production of long-chain and short-chain hydrocarbons derived from fatty acid metabolism along with the isoprenoid-derived hydrocarbons are reviewed. Also, the current limitations and future prospects in hydrocarbon-based biofuel production are discussed.

## Antioxidant effect of *Urtica dioica* on the stability of rapeseed oil during deep frying of French fries

Riyazi, S. S., and Asefi, N. *Int. J. Biosci.* 6: 20–28, 2015  
<http://dx.doi.org/10.12692/ijb/6.1.20-28>.

Antioxidant capacity of *Urtica dioica* extract used in dietology practice was determined by DPPH free radical method. Partially hydrophilic phenolic compounds are the most active compounds in plants. Therefore methanol was used as the

extraction agent. The total phenolics content were also measured and a strong correlation between these two variables was found. It is important to study about the use of natural antioxidants as alternatives to synthetic ones due to the possibility of carcinogenic effects of synthetic antioxidants. The aim of this study was to determine the antioxidant activity of methanolic extract of *Urtica dioica* leaves and comparing its antioxidant effect at levels of 100 and 800 ppm with synthetic antioxidant TBHQ at level of 100 ppm on the oxidative stability of rapeseed oil during deep frying of French fries. Results showed amount of phenolic compounds extracted by methanol were  $87.127 \pm 6.096$  mg gallic acid equivalent/g dry sample and antioxidant capacity was  $0.303 \pm 0.025$  mg. Results of peroxide value showed oil containing 100 ppm TBHQ had the lowest peroxide value ( $1.794$  meq  $O_2$ /Kg oil) after 96 h of deep frying. Results of acid, iodine and anisidine values indicated TBHQ has been more effective on stability of rapeseed oil after 48 h of deep frying. According to the results of sensory evaluation, samples fried in oil containing 100 ppm extract had the highest score throughout 48 h of frying. It is necessary to investigate higher concentrations of *Urtica dioica* leaves extract and compare with other synthetic antioxidants.

## Comparison of five analytical methods for the determination of peroxide value in oxidized ghee

Mehta, B. M., et al., *Food Chem.* 185: 449–453, 2015  
<http://dx.doi.org/10.1016/j.foodchem.2015.04.023>.

In the present study, a comparison of five peroxide analytical methods was performed using oxidized ghee. The

methods included the three iodometric titration viz. Bureau of Indian Standard (BIS), Association of Analytical Communities (AOAC) and American Oil Chemists' Society (AOCS), and two colorimetric methods, the ferrous xylenol orange (FOX) and ferric thiocyanate (International Dairy Federation, IDF) methods based on oxidation of iron. Six ghee samples were stored at  $80^\circ\text{C}$  to accelerate deterioration and sampled periodically (every 48h) for peroxides. Results were compared using the five methods for analysis as well as a flavor score (9 point hedonic scale). The correlation coefficients obtained using the different methods were in the order: FOX ( $-0.836$ ) > IDF ( $-0.821$ ) > AOCS ( $-0.798$ ) > AOAC ( $-0.795$ ) > BIS ( $-0.754$ ). Thus, among the five methods used for determination of peroxide value of ghee during storage, the highest coefficient of correlation was obtained for the FOX method. The high correlations between the FOX and flavor data indicated that FOX was the most suitable method tested to determine peroxide value in oxidized ghee.

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*Extracts & Distillates is compiled by Robert Moreau (US Department of Agriculture). Bryan Yeh (Intrexon) contributes references in the areas of industrial applications and synthetic biology. Extracts & Distillates is seeking a regular contributor with expertise in surfactants and detergents. If you are interested, please contact Kathy Heine at [kheine@aocs.org](mailto:kheine@aocs.org).*

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# Rapid detection of active bacteria in cold-pressed hemp seed oil

Edward F. Askew

Recent expansion of medical marijuana legalization at the state level across the United States has created a patchwork of both analytical methods and quality control systems for the processing of raw cannabis oil products. Such products can contain various amounts of the psychotropic drug  $\Delta$ -9-tetrahydrocannabinol, THC. In some states, the THC content in a cannabidiol oil extract is limited to no more than 3% by volume or active ingredient. With these limits in mind, cannabis oil produc-

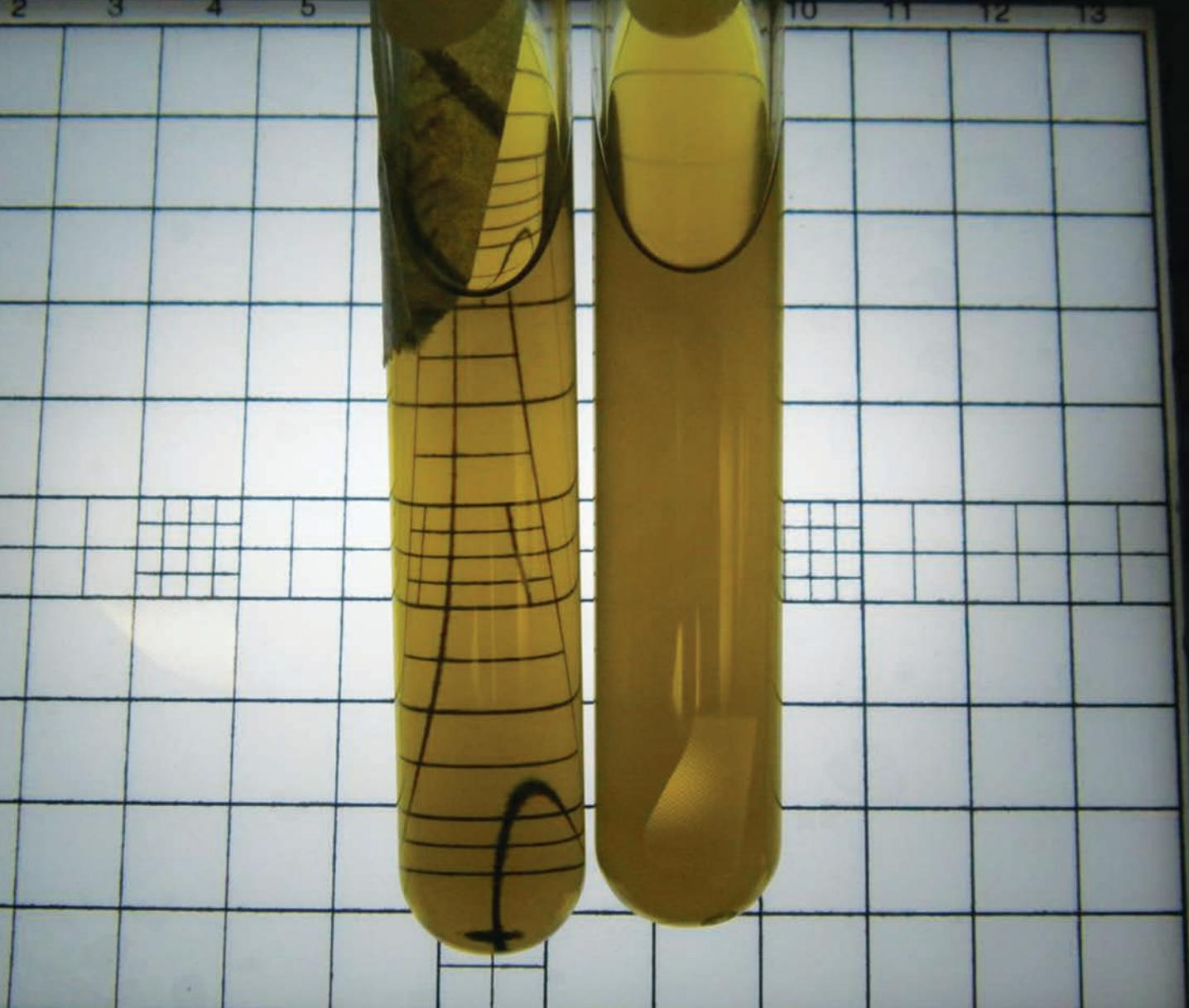
- When water is present, raw cannabis oil products can provide a medium in which pathogenic bacteria can grow. Such contamination could compromise safety in patients with the immune-compromising conditions these products are designed to treat.
- Unfortunately, most techniques used for bacteria analysis are too slow, too intrusive, or too expensive to use for production quality control.
- This article describes a technique based on oxygen uptake rate that can provide rapid bacteria analysis in six hours or less.

ers have utilized standard cold-press seed extractions or solvent extractions such as the use of butane or supercritical carbon dioxide. In all cases, the oil product produced is a mixture of fats, fatty acids, triglycerides, terpenes, and other materials. This organic mixture, if water is present, can produce a medium in which pathogenic bacteria can readily grow.

Work with cold-pressed oil palm product (Izah and Ohimain, 2013) has shown that opportunistic pathogens such as *Bacillus*, *Pseudomonas*, *Enterococcus* and *Staphylococcus* can colonize trace amounts of water at the oil/water interface. In this case, the bacteria were identified using older pour-plate bacteria methods (Akinrele, 1970) that required 48 to 72 hours to yield results. Unfortunately, production quality control requires a more rapid and reliable test that can be performed easily in a production laboratory. Molecular analysis using techniques such as real-time polymerase chain reaction (qPCR) and adenosine triphosphate (ATP) requires specialized laboratory space that is free from environmental contamination and isolated from any bacterial contaminants that may arise in the production area. Additionally, some of these techniques require expensive equipment and highly trained personnel, which makes them impractical for production quality control.

On the other hand, bacterial respiration through the use of oxygen uptake rate (OUR) (Askew, 2014) has been used to detect and quantify pathogenic bacteria in marine and freshwater beach samples rapidly with simplified instruments such as the GreenLight® (Baseline-Mocon). The OUR test measures the change in dissolved oxygen in a sample in which nutrient media, such as Tryptic Soy Broth, ISO Synthetic Sewage,





**FIG. 1.** Sterile and bacteria-inoculated samples of hemp seed oil

Enterococcosel Broth and other standard bacteria growth media, have been introduced. Once the bacteria have started to grow, both oxygen and food are metabolized and the drop in oxygen level is measured against a threshold endpoint. It has been shown that the time it takes to reach the threshold endpoint is proportional to the amount of active bacteria initially in the sample. The OUR test can produce results in as little as two hours for large amounts of active bacteria and in 12 hours for active bacteria levels of as low 100 CFU/100 mL.

The ability of the OUR test to measure active bacteria in cold-pressed hemp seed oil, which was used as a cannabidiol surrogate, was determined using a hemp seed oil matrix that had been inoculated with *E. coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. One  $\mu\text{L}$  loops of each bacterial inoculum was introduced to 10 mL of sterile hemp seed oil. In each case, incubation of the inoculated sample for 24 hours at 41°C produced a mixture that was cloudy compared to a sterile hemp seed oil sample. Figure 1 shows the comparison between a sterile sample and the cloudy sample that was produced by bacteria growth over the 24-hour period.

To confirm that the inoculated sample contained the bacteria of interest, 0.1  $\mu\text{L}$  aliquots were removed and suspended in 100 mL of sterile water. Confirmation for the three species of bacteria was performed through traditional methods, such as most probable number (MPN) IDEXX Laboratories Coli-Alert® and Pseudalert® and plate counts r-biopharm Compact Dry X-BC® over the required 24- to 48-hour testing. The OUR tests were then performed with 0.1  $\mu\text{L}$  aliquots of the inoculated hemp oil suspended in 100 mL of sterile water with bacterial growth medium added. The resulting samples were then transferred to GreenLight OUR vials and the dissolved oxygen levels were monitored. As the bacteria metabolized the growth medium and the oxygen in the sample vial, the signal increased. A threshold had been set based on prior work with beach water samples. The results are illustrated in Fig. 2 (page 398). The time to reach the threshold was proportional to the amount of active bacteria in the sample. Results were obtained within four hours.

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## Hemp Oil Bacteria Oxygen Uptake Rate (OUR) Analysis

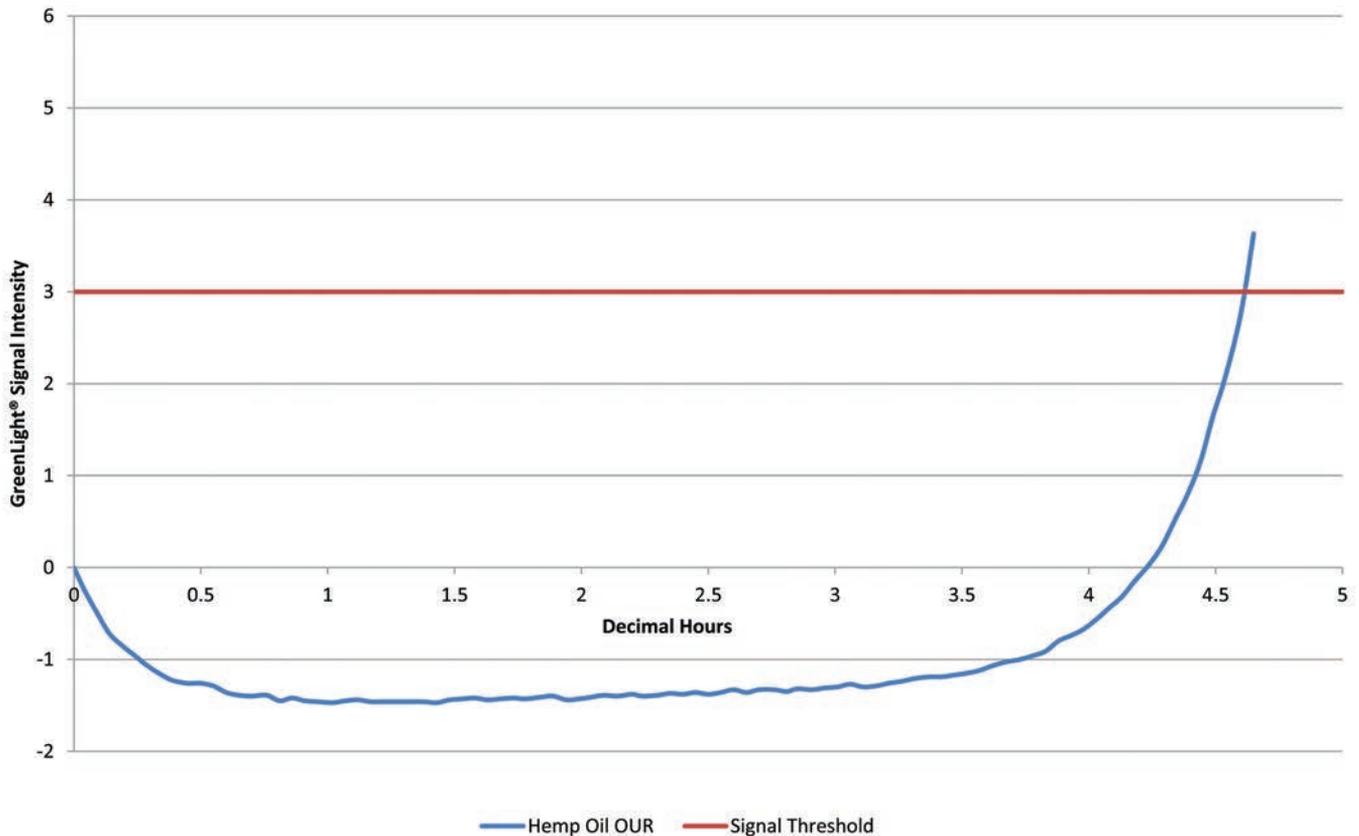


FIG. 2. OUR results from the GreenLight instrument

### Further reading

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- Izah, S. C. and E. I. Ohimain, Microbiological quality of crude palm oil produced by smallholder processors in the Niger Delta, Nigeria, *J. Microbiol. Biotech. Res.* 3: 30–36, 2013.

The OUR analysis can be used to detect active aerobic or facultative anaerobic bacteria rapidly at many steps of the cannabis oil production process. Examples include:

- detecting active bacteria in the raw cannabis oil as it comes off the process line;
- hygienic swab testing for active bacteria contamination of process control equipment and production areas;

- identifying active bacteria contamination in warehoused cannabis oil; and
- identifying active bacteria contamination in edible products manufactured using raw cannabis oil.

All of these tasks can be performed with simple equipment and staff trained in basic biological laboratory skills. This type of bacteria test will increase the patient safety of the cannabis oil product as it is used for the treatment of immune compromising disorders.

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*Edward F. Askew has worked in analytical testing and process control for more than 25 years, during which he provided pesticide analyses and enforcement for industry and state/federal regulators, water and wastewater analyses and enforcement for municipal and private sectors, and radiochemistry for federal/state regulators. In 2015, he helped form a Cannabinoid Technical Group that focuses on cannabis projects that include setting up testing laboratories at clients' growing, processing or food production facilities. Askew is currently a member of the American Oil Chemist Society (AOCS) Cannabis Expert Committee. He has been recognized by the AOCS as a technical expert on laboratory and production quality control and the development of baseline analytical test methods for cannabis products performance testing. He can be contacted at [efaskew@hotmail.com](mailto:efaskew@hotmail.com).*



# Five receive AOCS' highest honors

## 2015 Award of Merit and AOCS Fellows honored

**Nurhan Turgut Dunford**, an AOCS member since 1996, received the AOCS Award of Merit on Tuesday, May 5, at the AOCS Annual Meeting and Industry Showcases (AMIS) in Orlando, Florida, USA. The award recognizes leadership in technical, administrative, or special committees and AOCS activities, as well as outstanding service that has advanced AOCS' prestige, standing, or interests.



Dunford's commitment to AOCS and expertise as a professor of bioprocessing and oil and oilseed processing at Oklahoma State University have been invaluable to the Society over the past 20 years. She has served as a member at large, secretary/treasurer, vice chair and chair of the Processing Division, as well as on numerous Division and Society committees. In addition, she has organized and chaired symposia at every AOCS Annual Meeting since 2003 as well as serving as the general chair in 2012 and chair in 2013.

Her work with the AOCS Books and Special Publications Committee is also worthy of note. She has been both a member of the committee and a contributor, writing book chapters and functioning as co-editor of *Sunflower: Chemistry, Production, Processing, and Utilization*, which was published in 2015, and *Nutritionally Enhanced Oil and Oilseed Processing*, which appeared in 2004.

"A number of AOCS members quietly yet efficiently volunteer their time and talents to keep AOCS on the forefront of fats, oils, and lipids," said Gary R. List in his letter of nomination. "I believe that Nurhan Dunford qualifies for special recognition in view of her long and dedicated service to our Society."

"Through my volunteer activities, I expanded my professional network, gained many very close friends, and have had

mentors who are experts in their fields working at universities and in industry," noted Dunford.

"I would like to thank my friend and mentor, Gary List, for the nomination, the AOCS Merit Award committee members for their selection, and the AOCS staff for making Society volunteer work not a burden but fun."

## 2015 FELLOW INDUCTED

Four AOCS Fellows were inducted at the AMIS. They join a select group of veteran AOCS members whose achievements in science entitle them to exceptional recognition, or who have rendered unusually important service to the Society or to the profession. The four are Timothy G. Kemper, Alejandro G. Marangoni, Deland J. Myers, and Suk-Hoo (Steve) Yoon.

**Timothy G. Kemper**, global technical director, solvent extraction, for the Desmet Ballestra Group, is an authority on oilseed processing and solvent extraction. He is recognized for designing some of the largest and most efficient solvent extraction plants in the world, and he holds nine US patents related to oilseed crushing. The full scope of his work has enabled significant technical and commercial advances in oilseed extraction, and is documented in authoritative contributions to industry standard references, such as *Bailey's Industrial Oil and Fat Products* (6th edition).



Kemper has been an AOCS member for 27 years and has been active in committee work, Processing Division leadership, and on the Governing Board as president (2013), vice president (2012), treasurer (2008–2011), and member-at-large

CONTINUED ON NEXT PAGE

(2006–2007). He represents the highest ideals of professional leadership, scientific knowledge, and fellowship.



**Alejandro G. Marangoni**, a professor and Tier I Canada Research Chair Food, Health and Aging at the University of Guelph in Canada, primarily works on the physical properties of foods, particularly fat crystallization and structure. He is also co-editor-in-chief of *Current Opinion in Food Science*. His groundbreaking work on edible oleogels structured by a cellulose derivative has attracted worldwide attention from multinational companies. His

group has also discovered, characterized, and simulated the nanoscale structural level of fats. He has published over 300 refereed research articles, 51 book chapters, 12 books, and holds 14 issued patents. He received the Chang Award (Institute of Food Technologists 2014, AOCS 2013), Supelco/Nicholas Pelick–AOCS Research Award (2014), and was honored as one of the 10 most influential Hispanic Canadians in 2012.

He has been an AOCS member for 22 years. He has contributed to AOCS as a Governing Board member-at-large, an organizer of short courses, and in program development for many Divisions, in addition to serving as a book editor for AOCS Press.



**Deland J. Myers**, professor of cereal and food science at North Dakota State University in Fargo, USA, is well known as an expert on cereal and soy proteins. His study of the physicochemical, structural, and functional properties and application of cereal and food proteins in food systems began in product development at Pillsbury Co. His work at Iowa State University to develop soy protein adhesives for wood products

resulted in two patents; several companies now market soy-based adhesive formulations based on that work. All of these activities have helped promote new arenas for cereal and oilseed products through advances in protein processing technology.

Myers has been an AOCS member for 25 years and has contributed to the Society as an Annual Meeting session chair, by serving within the Protein and Co-Products Division leadership, and as technical chair of the meeting that marked the 100th anniversary of the Society. He also served as Governing Board president (2012), vice president (2011), member-at-large (2005–2010), and recently accepted the responsibility of chairing the AOCS Foundation.

**Suk-Hoo (Steve) Yoon**, a professor at Woosuk University in the Republic of Korea, is internationally recognized in the field of food chemistry and biotechnology. He has been a leader within the food science profession in Korea, especially in the area of food lipids. His research has furthered a better understanding of the chemistry of lipid oxidation, antioxidants, lipid analysis, the kinetics and mechanism of single cell oil fermentation, structured lipids, the development of novel lipid sources for food purposes, and the refining and processing of fats and oils. Further, he holds 13 patents and has published more than 180 papers, including review articles, and has written 26 book chapters.



Yoon has been an AOCS member for 36 years. He is a charter member and current chair of the Asian Section, and is a past chair of the Biotechnology Division. He served as president of the Korean Society of Food Science and Technology in 2012 and as president of the Korea Food Research Institute (2011–2013) while remaining active in the International Society of Biocatalysis and Agricultural Biotechnology (ISBAB). He received the Order of Science and Technology Merit from the President of Korea in 2012 and is a Fellow of the Institute of Food Technologists (2014) and ISBAB (2011). ■

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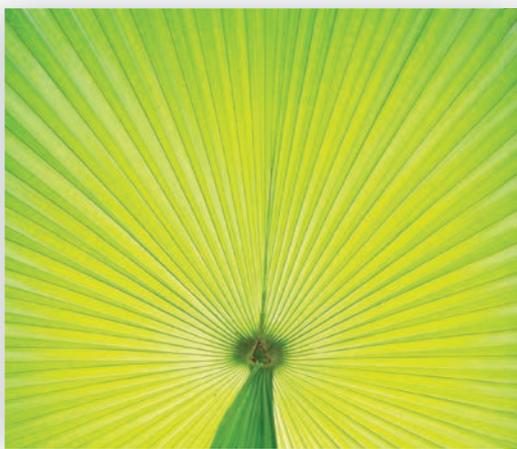
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