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Detecting parabens in environmental water samples

Researchers describe a green method using ionic liquids that can be used to detect parabens in environmental water samples.
A journey to standardization of bio-based surfactants in Europe

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New Methods

Five new methods accepted in 2016

- Ac 6-16 (Official Method) Extraction and Indirect Enzyme-Linked-Lectin-Assay (ELLA) Analysis of Soybean Agglutinin in Soybean Grain
- Cd 12c-16 (Standard Procedure) Accelerated Oxidation Test for the Determination of Oxidation Stability
- Cd 30-15 (Official Method) Analysis of 2- and 3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Oil-Based Emulsions
- Ce 12-16 (Official Method) Sterols and Stanols in Foods and Dietary Supplements Containing Added Phytosterols
- Ce 13-16 (Recommended Practice) Determination of Cyclopropenoic and Nutritional Fatty Acids in Cottonseed and Cottonseed Oil by Gas Chromatography

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The year 2016 witnessed Britain’s exit from the European Union, businessman Donald Trump’s election as US President, the announcement of several corporate mega-mergers—and the approval of five new AOCS methods. Although perhaps not as momentous to the world-at-large as the other events, the new AOCS methods fill critical gaps in the analysis of edible oils and oilseeds. Since the 1920s, AOCS has identified and validated analytical methods crucial for the processing, trade, utilization, and evaluation of fats, oils, lipids, and related products. The new methods join more than 450 other AOCS Official Methods, Recommended Practices, and Standard Procedures in the *Official Methods and Recommended Practices of the AOCS, 7th* edition, released in March of this year (http://tinyurl.com/AOCS-methods-book).

**BECOMING OFFICIAL**

To join their esteemed colleagues in the *Official Methods and Recommended Practices of the AOCS, 7th* edition, the five newest methods followed a well-established procedure. The process begins with the submission of a proposal. “Anybody can propose a new method, even the AOCS itself,” says Richard Cantrill, chief science officer at AOCS. “We identify methods mainly from industry need. We also pick them up from journal articles, or collect them in harmonization activities with other organizations.” According to Cantrill, the proposer of a new method is asked to make a presentation in one of the methods sessions at the AOCS Annual Meeting and Industry Showcases. “It’s a bit gladiatorial,” says Cantrill. “The audience basically gives thumbs up or thumbs down, or asks for more detail. It can be a bit unnerving, so anyone making a presentation has to have enough data to show that they’ve actually worked the method out.”

The proposal is then submitted to an expert panel or a subcommittee of the AOCS Uniform Methods Committee, which decides whether the method has been sufficiently validated to warrant a collaborative study. International guidelines require collaborative studies to include a minimum of eight expert laboratories, preferably domestic and international. The Uniform Methods Committee and AOCS staff select labs that have expertise in method development and in the particular topic to participate in a collaborative study.

Samples that have been sourced and prepared to ensure homogeneity are then delivered to participating labs, with a deadline for returning results. After receiving data from all labs involved in the collaborative study, AOCS staff run a statistical analysis on the data. For each sample, an overall mean of laboratory values, repeatability standard deviation \( s(r) \), reproducibility standard deviation \( s(R) \), and other parameters are calculated. Repeatability refers to the ability of the same lab to obtain similar values for replicate samples using the same instrument, under the same...
conditions, during a short period of time. In contrast, reproducibility compares results for the same samples between labs.

“Repeatability tells you how well you’re doing within your lab, while reproducibility tells you how well a whole cohort of labs is doing when they perform that method,” says Cantrill. “Generally the reproducibility standard deviation is larger than the repeatability standard deviation.” The repeatability relative standard deviation [RSD(r)] and reproducibility relative standard deviation [RSD(R)] express the standard deviation as a percentage of the mean value. “You’re looking for a 1–2% spread, but as you get down toward the limit of detection, you might end up with a 30% spread,” says Cantrill. And finally, the repeatability value (r) and the reproducibility value (R) reflect the 99% confidence interval for the data.

The data and statistical analysis are then presented to the Uniform Methods Committee, which can reject the method, or approve it by a two-thirds majority vote. If the method is rejected, the committee may make recommendations to change parts of the method and conduct another collaborative study. According to Cantrill, the data evaluation is left to the committee’s discretion, with no specified cutoff values for reproducibility or repeatability. “If the method is loosely written and not very easy to follow, you usually get really bizarre results back, so it’s sort of self-limiting,” he says.

After a method is officially adopted, it is named according to AOCS conventions and included in the *Official Methods and Recommended Practices of the AOCS* (see “What’s in a Name” on page 8). There are three types of AOCS methods: Official Methods, Recommended Practices, and Standard Procedures. An Official Method has been validated and approved by the process described above. A Standard Procedure is a method that relies on a specific apparatus in accordance with the manufacturer’s instructions. Unlike Official Methods, Standard Procedures can be vendor-specific. Standard Procedures are validated and approved by the same procedure as that for Official Methods. On the other hand, Recommended Practices are methods that may be of interest or value, but they do not have enough validation data to qualify as an Official Method. A Recommended Practice may or may not have been subjected to a collaborative study. In some cases, a collaborative study may reveal data variation that is unacceptable for an Official Method, but the method may still be of value for simple, rapid, or qualitative analyses.

**OFFICIAL METHOD AC 6-16: EXTRACTION AND INDIRECT ENZYME-LINKED-LECTIN-ASSAY (ELLA) ANALYSIS OF SOYBEAN AGGLUTININ IN SOYBEAN GRAIN**

Soybean agglutinin (SBA) is a carbohydrate-binding protein, or lectin, that decreases the growth rate of monogastric animals, such as chickens and swine, that consume raw soybean seeds. “Soybean agglutinin is considered an anti-nutrient, and it is measured in all soybean biotech products as part of the safety assessment for regulatory approvals,” says Elisa Leyva-Guerrero, a plant biochemist at Monsanto (St. Louis, MO, USA) who helped develop Official Method Ac 6-16. Heat from cooking or other processing destroys most of the SBA in raw soybeans.

Since the 1950s, scientists have quantified SBA with a hemagglutination technique that requires rabbit red blood cells. As a lectin, SBA can bind to polysaccharides on the surfaces of red blood cells, causing the cells to clump together, or agglutinate. However, the hemagglutination method is costly, time-consuming, not very accurate, and has arbitrary units (hemagglutinating units), says Leyva-Guerrero.

The new method Ac 6-16 uses an enzyme-linked lectin assay (ELLA) to quantify SBA. The technique is analogous to the well-known enzyme-linked immunosorbent assay (ELISA), but uses carbohydrates to capture and detect SBA, rather than antibodies. Specifically, the carbohydrate N-acetylgalactosamine (GalNAc) is linked to polyacrylamide (PAA) to immobilize the carbohydrate within the wells of a microtiter plate. When soybean extract is added to a well, SBA in the extract binds to the immobilized carbohydrate. Then, a biotinylated version of GalNAc-PAA (GalNAc-PAA-Biotin) is added, which also binds to SBA, forming a “sandwich.” Washing steps remove any unbound GalNAc-PAA-Biotin. To detect the immobilized GalNAc-PAA-Biotin (and associated SBA), researchers add a conjugate of neutravidin (a protein that binds specifically to biotin) and horseradish peroxidase (HRP). Upon addition of 3,3’,5,5’-tetramethylbenzidine (TMB), a chromogenic substrate for HRP, a yellow pigment is pro-
duced that absorbs light at 450 nm. When the microtiter plate is placed in a plate reader, the absorbance at 450 nm of multiple samples and standards can be measured simultaneously. By comparison to a standard curve of known SBA values, the researchers can calculate the mg of SBA per g of soybean tissue.

With a 2-hour incubation procedure, ELLA exhibits sensitivity in the μg/mL range—more than sufficient to detect SBA in soybean, which is expressed in the mg/mL range (Breeze, M. L., et al., http://dx.doi.org/10.1007/s11746-015-2679-3, 2015). The new method demonstrates a linear response to purified SBA over one order of magnitude. Another advantage is that all reagents required for the method, including GalNAc-PAA and GalNAc-PAA-Biotin) are commercially available. Leyva-Guerrero and her colleagues used the validated ELLA method to quantify SBA in nine commercial soybean varieties introduced between 1972 and 2008 (Breeze, M. L., et al., http://dx.doi.org/10.1007/s11746-015-2679-3, 2015). The study revealed that the concentration of SBA ranged from 2.03 to 2.92 mg/g and varied with soybean genotype and environment.

“The ELLA method can measure SBA with increased accuracy, non-arbitrary units (mg lectin/g seed), and decreased cost and time in comparison to the hemagglutination method,” says Leyva-Guerrero. She notes that Official Method Ac 6-16 was developed and validated with the support of the Analytical Excellence through Industry Collaboration (AEIC; https://aeic-biotech.org) Composition Working Group, whose members represent major agricultural biotech companies and contract research organizations.

OFFICIAL METHOD CD 30-15: ANALYSIS OF 2- AND 3-MCPD FATTY ACID ESTERS AND GLYCIDYL FATTY ACID ESTERS IN OIL-BASED EMULSIONS

Monochloropropane-1,2-diol (MCPD) esters and glycidyl esters are process contaminants formed during the high-temperature deodorization step of edible oil refining (Cassiday, L., Inform 27, 6–11, 2016) (Fig. 1). In recent years, these contaminants have come under increased scrutiny by food safety organizations such as Germany’s Federal Institute for Risk Assessment (BfR) and the European Food Safety Authority (EFSA) after free MCPD and glycidol were linked to cancer, infertility, and other health problems in animal studies. 2- and 3-MCPD esters and glycidyl esters have been detected in refined oils (mainly palm), as well as in oil-containing foods such as bread, margarine, French fries, baby food, and infant formula.

Official Method Cd 30-15 joins three other AOCS Official Methods for the simultaneous analysis of 2- and 3-MCPD esters and glycidyl esters (Cd 29a-13, Cd 29b-13, and Cd 29c-13). “The original methods were only for fats and oils, mainly vegetable oils,” says Cantrill. “Cd 30-15 is AOCS’s first method for analyzing 2- and 3-MCPD esters and glycidyl esters in fatty foods.” Cd 30-15 is an extraction procedure for isolating 2- and 3-MCPD and glycidyl esters from oil-based emulsions such as spreads, margarines, dressings, and mayonnaise. After extraction, any of the previously published methods (Cd 29a-

What’s in a name?

Have you ever wondered how an AOCS method gets its name (Ac 6-16, Cd 39-15, etc.)?

• The capital letter refers to the section of the Official Methods and Recommended Practices of the AOCS in which the method appears:
  Vegetable oil source materials (Section A)
  Oilseed by-products (Section B)
  Commercial fats and oils (Section C)
  Soap and Synthetic Detergents (Section D)
  Glycerin (Section E)
  Sulfonated and Sulfated Oils (Section F)
  Soap stocks (Section G)
  Specifications for Reagents, and Solvents and Apparatus (Section H)
  Lecithin (Section J)
  Evaluation and Design of Test Methods (Section M)
  Analytical Guidelines for Testing Industrial Oils and Derivatives (Section S)
  Test Methods for Industrial Oils and Derivatives (Section T)

• The lower-case letter designates a group of related methodologies within a section. For example, “Ab” methods all involve the analysis of peanuts as vegetable oil source materials.

• The first number (before the dash) refers to the number of the method within the group of related methodologies. For example, Ab 1, Ab 2, Ab 3, etc.

• The second number (after the dash) indicates the year the method was first published. For example, Ac 6-16 was published in 2016, whereas Aa 1-38 was introduced in 1938.

To order the Official Methods and Recommended Practices of the AOCS, 7th Edition, or to purchase individual methods, visit the Methods section of the AOCS website (https://www.aocs.org/attain-lab-services/methods).
Cd 13, Cd 29b-13, or Cd 29c-13) may be used to quantify these process contaminants.

In Cd 30-15, the food sample is first homogenized in a solvent consisting of heptane and methyl tert-butyl ether, and then incubated in an ultrasonic bath. Next, a liquid-liquid extraction is performed to purify lipophilic substances in the sample. After evaporation of organic solvents, the lipophilic substances (including 2- and 3-MCPD and glycidyl esters) can be analyzed by one of the previously published AOCS Official Methods. Although the methods differ in experimental details, they all involve the chemical cleavage of esters from 2- and 3-MCPD and measurement of the free MCPD by gas chromatography/mass spectrometry (GC/MS). Two of the methods (Cd 29a-13 and Cd 29b-13) convert glycidyl esters to 3-monobromopropanediol (3-MBPD) prior to GC/MS analysis. The third (Cd 29c-13) involves converting 2- and 3-MCPD and glycidyl esters to 3-MCPD in the presence and absence of chloride (glycidol cannot form 3-MCPD without chloride).

The extraction procedure showed good recovery of 2- and 3-MCPD and glycidyl esters and high sensitivity (limit of detection, 0.04 and 0.05 mg/kg for MCPD and glycidyl esters, respectively), in addition to satisfactory repeatability and reproducibility (Ermacora, A., and Hrnčířík, K., http://dx.doi.org/10.1080/19440049.2014.905712, 2014).

OFFICIAL METHOD CE 12-16: STEROLS AND STANOLS IN FOODS AND DIETARY SUPPLEMENTS CONTAINING ADDED PHYTOSTEROLS

Plant sterols and stanols, collectively known as phytosterols, are cholesterol-like molecules in plants that have been shown to reduce serum total and low-density lipoprotein (LDL) cholesterol levels in humans who consume them. Because of their potential to reduce the risk of cardiovascular disease, phytosterols are added to many foods such as margarines and other spreads, salad dressings, and snack bars, as well as dietary supplements. The US Food and Drug Administration (FDA) allows food and supplement manufacturers to make health claims on the relationship between phytosterols and a reduced risk of coronary heart disease, provided that the products contain specified amounts of the five major phytosterols that have shown beneficial effects (campesterol, campestanol, stigmasterol, β-sitosterol, and sitostanol; Fig. 2, page 10).
According to Cantrill, Official Method Ce 12-16 arose from a collaboration between Cargill and the FDA. “Sterols and stanols are commonly included in margarines and dietary supplements, so Official Method Ce 12-16 is a test to find out whether they have the correct amounts of sterols and stanols as claimed on the label,” he says. Previous methods for phytosterol analysis were limited in various aspects, such as a lack of validation for stanol quantification, limited range or accuracy, or unsuitability for the analysis of dietary supplements (Srigley, C. T., and Haile, E. A., http://dx.doi.org/10.1016/j.jfca.2015.01.008, 2015).

Method Ce 12-16 can determine total free sterols/stanols and total steryl/stanol esters, as well as quantify each of the five major phytosterols that are the subject of the FDA’s health claim. Ce 12-16 provides three protocols for extracting phytosterols from different matrices (sterol/stanol concentrates, steryl/stanol ester concentrates, foods, and dietary supplements). Each protocol derivatizes the phytosterols to trimethylsilyl (TMS) ethers so that they may be separated on a capillary GC column, detected by a flame ionization detector (FID), and identified by their retention times. The method uses eicoprostanol as an internal standard.

Using the method, researchers determined that 25 analyzed samples, including spreads, beverages, baked goods, and dietary chews, had total phytosterol contents that varied from 0.2 to 55.2 g/100 g (Srigley, C. T., and Haile, E. A., http://dx.doi.org/10.1016/j.jfca.2015.01.008, 2015). Total phytosterol contents ranged from 83% to 137% of the amounts declared on labels. The limit of detection (LOD) and limit of quantitation (LOQ) for an individual phytosterol were 0.3 mg/100 g and 1 mg/100 g, respectively.

RECOMMENDED PRACTICE CE 13-16: DETERMINATION OF CYCLOPROPENOIC AND NUTRITIONAL FATTY ACIDS IN COTTONSEED AND COTTONSEED OIL BY GAS CHROMATOGRAPHY

Cotton plants of the genus Gossypium are cultivated primarily for their textile fibers. “However, cotton products are also consumed by humans and animals,” says Barb Mitchell, staff scientist at Covance Labs, Inc. (Madison, Wisconsin, USA), who helped develop Recommended Practice Ce 13-16. Cottonseed oil has been used as a cooking oil and in foods such as mayonnaise and salad dressing, and cottonseed meal is included in animal feed. “As new cottonseed varieties are developed through biotechnology, it is necessary to assess their safety,” says Mitchell.

According to Mitchell, the Organization for Economic Co-operation and Development (OECD) recommends the analysis of fatty acid profiles in cottonseed—both nutritional fatty acids and the anti-nutritive cyclopropenoid fatty acids. “Cyclopropenoid fatty acids in cottonseed include malvalic acid, sterculic acid, and dihydrosterculic acid, which have been shown to have unfavorable health effects in livestock,” says Mitchell. Most cyclopropenoid fatty acids are removed during the deodorization step of oil refining, but the fatty acids can be a problem for producers of cold-pressed cottonseed oil.

Historically, the determination of nutritional fatty acids and of cyclopropenoid fatty acids required two separate analyses. Recommended Practice Ce 13-16 combines the two analyses into a single GC procedure. First, the triacylglycerols from cottonseed or cottonseed oil are converted to fatty acid methyl esters by base transesterification using sodium methoxide. Then, the individual esters are analyzed by GC using a polyethylene glycol stationary phase with an FID.

“The biggest challenge was to adjust the GC conditions to get the best resolution between both malvalic and stearic acids and dihydrosterculic and α-linolenic acids,” says Mitchell. “What worked better for one pair was worse for the other pair.” However, the researchers eventually determined GC conditions that allowed an adequate separation of all compounds. The LOQ for various nutritional and cyclopropenoid fatty acids ranged from 0.001 to 0.012 mg/mL (Mitchell, B., et al., http://dx.doi.org/10.1007/s11746-015-2669-5, 2015).

STANDARD PROCEDURE CD 12C-16: ACCELERATED OXIDATION TEST FOR THE DETERMINATION OF THE OXIDATION STABILITY OF FOODS, OILS, AND FATS USING THE OXITEST OXIDATION TEST REACTOR

Lipid oxidation is a major factor that limits the shelf life of foods containing fats and oils (Cassiday, L., Inform 26, 406–411, 2015). Various methods exist for assessing the rate of lipid oxidation in foods. However, these techniques require the fat to be extracted from food samples before oxidation tests can be per-
formed. In contrast, the Oxitest instrument (VELP Scientifica; Usmate, Italy) can analyze fat oxidation in whole food samples, providing a simpler and more rapid method (Fig. 3).

Standard Procedure Cd 12c-16 details how to use the Oxitest Oxidation Test Reactor to analyze the oxidative stability of whole food samples. Two samples can be analyzed simultaneously on the same instrument. A food sample, which can be liquid, solid, or doughy, is placed in one of two oxidation chambers, where it is subjected to accelerated oxidation conditions of high temperature (up to 110°C) and high oxygen pressure (up to 8 bar). In this way, lipid oxidation can be observed over a shortened time period (hours) compared with the days, weeks, or months required for the food to naturally become rancid. By monitoring changes in absolute pressure within the chamber, the Oxitest instrument measures the oxygen uptake of reactive components in the food. The instrument generates a value called the Induction Period (IP), which refers to the time required for a sample to show a sudden increase in the rate of oxidation. The longer the IP, the more resistant the sample is to oxidation. Cd 12c-16 can be used for a wide range of sample types with at least 2–4% fat content, including meat, oils, mayonnaise, and baked goods.

Researchers used the Oxitest method to analyze the oxidative stability of several extra virgin olive oils that came from two regions of Italy (Caruso, M. C., et al., Inform 28, 26–29, 2017). They found a strong correlation between the total content of polyphenols (which are natural antioxidants) in the olive oil and oxidation stability, as measured by IP. The IP values for all of the investigated oils ranged from 20 to 78 hours. The data did not indicate a direct correlation between geographical origin of the olive oil and IP value.

Although the five new AOCS methods may not have made the top news headlines of 2016, they will certainly be appreciated by members of the fats and oils community. The availability of reliable, accurate, validated methods will simplify and accelerate research on fats, oils, and the foods that contain them.

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In humans, brain function requires a lot of energy: roughly 22% of the body’s daily energy intake. The brain’s main fuel is glucose, which is normally abundantly available both from dietary carbohydrates and from glucose made in the body via gluconeogenesis. The problem for the aging brain is not glucose supply, but rather its deteriorating ability to take up and use glucose available in the blood. The tendency as we age is to become more sedentary and gain a little weight, which makes it harder to metabolize glucose. This often leads to mild glucose intolerance, which commonly progresses to type two diabetes—a major risk factor for cognitive decline during aging. Cognitive decline during aging can remain relatively innocuous but may also progress to Alzheimer’s disease (AD), in which worsening memory problems leave people incapable of caring for themselves and managing on a day-to-day basis.

Glucose is the brain’s main fuel but not its only fuel. Like other organs, the brain has a seamless back-up fuel when glucose supply is insufficient, such as during fasting, starvation, strenuous exercise, or malnutrition. However, unlike other organs which use free fatty acids to replace low glucose supply,
the brain uses ketones (or ketone bodies) as a back-up for glucose. The ketones that replace glucose for the brain are β-hydroxybutyrate (β-HB) and acetoacetate (AcAc). Our research group has been studying brain glucose and ketone metabolism in healthy older people with no memory problems, and in those who have progressed to AD or who have mild cognitive impairment (MCI), an intermediary stage just before AD. We use positron emission tomography (PET) imaging to measure brain energy metabolism. Brain glucose PET imaging is quite common, but we were the first to develop brain ketone PET imaging. By administering the ketone tracer followed an hour later by the glucose tracer, it is possible to get a more complete picture of brain fuel metabolism. The results have been very interesting. This dual-tracer technique demonstrates that there is a critical difference between brain glucose and brain ketone metabolism during aging, a difference that is the basis for a new treatment strategy in AD.

Glucose uptake by the brain deteriorates in AD, especially in the parietal cortex (above the ears). Memory fails because more and more brain cells die, and the general assumption has been that brain glucose uptake declines in AD because the need for glucose has decreased due to loss of brain cells. This makes perfect sense except for one thing: it does not account for the fact that brain glucose uptake is actually already decreasing in people at risk for AD but before the onset of memory problems.

**BRAIN FUEL PROBLEMS BEFORE THE ONSET OF AD**

There are several conditions in which higher genetic or lifestyle risk of AD causes lower brain glucose uptake long before the onset of memory problems or cognitive symptoms. The most serious is a mutation in the presenilin-1 gene which essentially guarantees AD. The E4 allele of apolipoprotein E also increases AD risk, but not nearly as much as having the mutated presenilin-1 gene. Maternal family history of AD also increases AD risk. Type 2 diabetes and insulin resistance are among the most important lifestyle-based risk factors for AD. All of these conditions increase the risk of AD, and in each there are problems with brain glucose uptake decades before the onset of memory problems, when cognitive test results are still normal. If lower brain glucose uptake were really only a consequence of brain cells dying in AD, how is it that brain glucose uptake is already lower before the onset of symptoms? We interpret these results to mean that problems with brain energy metabolism could contribute to the development and/or progression of AD; the brain glucose problem undoubtedly gets worse as brain cells die and the disease progresses, but it also seems to contribute to AD’s onset because this problem is sometimes present decades before the disease starts.

We therefore propose that a vicious cycle can develop: Brain glucose uptake starts to decline, which leads to gradual brain energy deprivation, deteriorating brain function, further decline in demand for glucose, and further cognitive decline (Fig. 1.).

**KETONES: THE BRAIN’S PHYSIOLOGICAL ALTERNATIVE FUEL**

Ketones are commonly associated with uncontrolled type 1 diabetes, so the medical community is often reticent to con-
consider them to be physiologically important as a brain fuel. Important progress in demonstrating the necessary function of ketones was made in the 1960s and 1970s during medically supervised voluntary starvation studies lasting 40–60 days. These studies unequivocally demonstrated that the ketones \( \beta\text{-HB} \) and AcAc were important for the brain, as these ketones could meet up to 80% of the adult brain’s energy requirements during starvation.

Ketones are produced from fatty acids that are released from body fat when insulin levels are reduced. Fasting reduces glucose and insulin, and permits body fat stores to release free fatty acids, which are the principal back-up fuel for all organs except the brain. Free fatty acids are converted to ketones in the liver, but since the liver cannot use ketones as a fuel, they are released into the circulation where they reach the brain. Other organs can potentially use ketones as fuels during fasting or starvation but, in practice, they are used principally by the brain. Sedentary lifestyles and excessive intake of simple sugars commonly lead to slower insulin clearance and chronic hyperinsulinemia, which compromise not only glucose metabolism but also ketone production. Consequently, the hyperinsulinemia of type 2 diabetes puts the aging brain in double jeopardy as the brain becomes 1) less able to use glucose, and 2) blockage by chronic hyperinsulinemia causes fewer ketones to be produced.

**BRAIN KETONE UPTAKE IN EARLY AD**

One can debate the importance of declining brain glucose uptake for the onset of AD; indeed this debate may never be fully resolved. We took the approach that doing brain glucose and ketone PET on the same person on the same day would allow us to compare the uptakes of these two brain fuels. This would in turn permit us to assess the value of testing the ability of ketones to rescue brain fuel supply in AD. Since brain ketone uptake uses a different transporter than glucose, and a different access point into mitochondria to make the fuel for cells (ATP), our approach would show whether the brain uptakes of its two most important fuels differed in people with or at risk of AD. If brain ketone uptake was more-or-less normal but brain glucose uptake was defective, we would assume that the brain cells must still be in fairly good shape but just not able to use glucose; in this case, brain fuel rescue would be a possibility. However, if the same pattern of failing brain glucose uptake also affected ketones, it would be more likely that brain cell function was severely impaired on several fronts, and brain fuel rescue by ketones would be unlikely.

We have been studying this question for nearly 10 years and have confirmed, as expected, that our AD patients had about 14% lower brain glucose uptake, a value matching what has been reported elsewhere on many occasions. However, brain ketone uptake was not significantly different in the brain as a whole or in any brain region in AD versus the controls. Furthermore, ketone utilization by the brain was highly correlated to plasma ketones, and the slope of this relationship did differ between controls and early AD. This suggests that the problem with brain fuel metabolism early in AD is specific to glucose, because both brain uptake and metabolism of ketones was still normal. That’s both good news and bad news: good news because perhaps brain fuel rescue is possible; bad news because ketones rarely supply more than 5% of brain energy requirements unless a person fasts for more than a day. Hence, we realized that it would be a challenge to provide the brain with ketones in the quantities necessary to overcome the brain glucose deficit in AD.

For us, the next step was to assess whether the brains of people with AD could use additional ketones if enough were provided to meet 10–15% of their brains’ energy requirements. Although fasting and a very high ketogenic diet can be used to stimulate ketogenesis, it is difficult to get good compliance from people who are asked to fast or radically change their diet for more than 8 hours! We took an alternative approach by using an MCT supplement.

**MCT: A GOOD WAY TO PRODUCE KETONES**

MCT are well-known in clinical nutrition and are much more ketogenic than the more common long-chain fats in the diet. Indeed, breast-fed infants achieve relatively sustained mild ketosis because they are consuming medium-chain fatty acids present naturally in breast milk. Medium-chain fatty acids gain rapid and relatively direct access to the liver because they are absorbed through the portal vein rather than via lymph into the general circulation like long-chain fats. MCT are also more easily beta-oxidized than long-chain fatty acids, which contributes to their ketogenic efficacy.

There is a strong positive correlation between the oral dose of MCT consumed and the maximal plasma ketone level achieved (Fig. 2.), so it is possible to predict the ketogenic response to an oral dose of MCT. Among common dietary oils, coconut and palm kernel oils contain the most medium-chain fatty acids: octanoic (caprylic; C8) acid and decanoic (capric; C10) acid. These two fatty acids are present in both oils at about 6–10%. One-day metabolic studies in humans show that C8 is considerably more ketogenic than C10. Coconut and palm kernel oils also contain lauric acid (C12), but its ketogenic effect is not well known.

- FIG. 2. Direct relationship between increasing oral dose of medium chain triglyceride and maximal plasma ketone (\( \beta\text{-hydroxybutyrate} \) \( \beta\text{-HB} \)) \( \gamma = 0.01X + 0.08; R^2=0.97; p<0.0001 \). See Cunnane et al. (2016) for details.
KETONES HELP TREAT COGNITIVE PROBLEMS

Our PET study results showing that brain ketone uptake is still normal in AD form the basis for subsequent clinical studies with an MCT supplement. The concept of using a ketogenic intervention to rescue brain energy metabolism in older people is relatively recent. However, ketogenic treatments of neurological conditions, including the very high-fat ketogenic diet for epilepsy, are not new. Oral MCT help control intractable epilepsy in children, and also improve cognitive outcomes during controlled experimental hypoglycemia caused by insulin infusion into the blood. Small studies by other researchers have shown that the very high-fat ketogenic diet has a beneficial effect on cognitive and heart health in both MCI and AD. Such reports demonstrate that ketones can support brain function even when plasma glucose is severely reduced. However, the tolerability of a ketogenic intervention for long-term use in older people remains an issue.

SAFETY OF MEDIUM-CHAIN TRIGLYCERIDES

MCT have a strong safety record in all species studied, including humans. They are well-tolerated at doses up to 0.5 g/kg/d (or about 30 g/d); above that, gastrointestinal side effects, including diarrhea, can be expected to increase in some people as the dose increases. However, these side effects can be somewhat mitigated if the final dose is achieved gradually. Concern exists about whether MCT, which are saturated fats, increase cardiovascular risk factors or body weight, but there is no evidence that this is the case; indeed, they are quite widely used for weight loss. Several products based on MCT are now available over the counter or by prescription in the United States and in Europe. The arrival of these products was greatly stimulated by reports from Accera Ltd. that the MCT tricaprylin (C8 triglyceride) had beneficial effects on cognitive outcomes in mild-moderate AD after a single dose or with regular consumption over several months.

PERSPECTIVE FOR THE FUTURE

Several studies show that brain ketone uptake is still normal early in AD, which supports the plausibility of assessing a “keto-neurotherapeutic” strategy. The goal is to determine whether a ketogenic treatment can help the brain overcome or bypass the creeping decline in brain glucose uptake and metabolism with age, thereby delaying brain energy exhaustion and decreasing the risk of AD. Some studies have already shown promise toward this goal, but a recent Phase 3 trial of tricaprylin in mild-moderate AD was negative—possibly because the minimum effective dose of MCT was not achieved.

Ketone PET imaging of the brain has been essential for our group to estimate the therapeutic target for MCT for a Phase 3 trial that is presently in preparation. This imaging technique will be a key tool in assessing whether ketones improve cognitive outcomes in AD due to brain energy rescue, or perhaps other mechanisms, such as a protective effect against senile plaques (beta-amyloid) or neuroinflammation. Perhaps brain energy rescue could be combined with current therapies that focus on maintaining brain levels of the neurotransmitter, acetylcholine. Large sums continue to be invested in AD treatments aimed at blocking beta-amyloid in the brain but this approach has not been successful—perhaps in part because the brain’s energy problem is not being addressed. MCT and/or a very high fat ketogenic diet are not the only way to address this problem. There are now multiple ways that brain ketone supply can be increased using ketone esters or salts, caloric restriction, or intermittent fasting, all of which provide options to validate the utility of keto-neurotherapeutics.

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Further reading
Detecting parabens in environmental water samples

Md Saleh Noorashikin, Farhanini Yusoff, Karthi Suresh, and Ruzita Ahmad

Parabens (alkyl esters of p-hydroxybenzoic acid) are a class of preservatives used in more than 22,000 personal care products (Andersen, 2008). Due to their broad antimicrobial spectrum, non-volatility, good stability, and effectiveness over a wide pH range, parabens such as methyparaben, ethylparaben, propylparaben, and butylparaben, are widely used—often in combination—to prevent microbial growth in toothpaste, sunscreen, shampoo, hair gel, face lotion, foundation, lipstick, and other cosmetics (Fei et al., 2011).

• Parabens are used in more than 22,000 personal care products, where they are used to prevent microbial growth.

• Although parabens protect food and products, making them last longer, exposure to parabens could harm human cells—particularly if they are discharged without proper treatment.

• This article describes a green method that can be used to detect parabens in environmental water samples.

PARABENS IN THE BODY AND THE ENVIRONMENT

The daily usage of paraben-containing cosmetic products is estimated to be 17.76 g for adults and 378 mg for infants (Hou et al., 2014), while the average daily total exposure to parabens is estimated to be 76 mg, with cosmetic and personal care products accounting for 50 mg, 25 mg from pharmaceutical products, and 1 mg from food (Gosens et al., 2014).

Although parabens protect food and many other products from microbial contamination, concerns have been raised about the effects of paraben exposure on human cells. Parabens have been found in the breast tissue of breast cancer patients, and studies conducted by the National Health and Nutrition Examination Survey (NHANES) USA in 2010, showed that parabens can disrupt the function of the endocrine system by mimicking estrogen, which can cause the human breast to generate tumor cells.

Parabens that enter the human body through the skin are metabolized by keratinocyte carboxylesterases, and the conjugated metabolites are excreted via urine. It is therefore not surprising that parabens have been found in rivers, streams, and effluent from wastewater treatment plants (Kirchhof & de Gannes, 2013). Consequently, being able to detect and monitor such compounds in environmental waters is becoming increasingly important.

IONIC LIQUID IN PARABEN-CONTAINING PRODUCTS

Ionic liquids (ILs) are a new class of solvent with an ionic structure that synergistically contributes to the increase in solubility of biomolecules in water. ILs can enhance the solubility of hydrophobic substances in aqueous media, thus they are widely used in the formulation of food, pharmaceutical, and personal care products.

Food and pharmaceutical industries are also interested in using ILs to separate and purify parabens for two reasons: 1. ILs have properties that are
advantageous for extraction, such as good thermal stability and extractability of organic and inorganic ions, and the ability to select for optimal viscosity and miscibility with either water or organic solvents. (Flieger & Czajkowska-Żelazko, 2011). 2. Conventional processes such as ion-exchange chromatography and liquid membrane extractions are expensive and environmentally hazardous (Ruiz-Aceituno et al., 2013), so ILs represent a new, greener alternative in the extraction of parabens.

**IONIC LIQUIDS IN THE DETECTION OF PARABENS**

ILs do not have measurable vapor pressure, so they cause zero emission of volatile organic compounds (VOC’s). For this reason, ILs are commonly used in the chemistry field, not only to detect parabens, but also as a biological reaction medium to stabilize enzymes during pharmaceutical synthesis and to remove metal ions and purify gas in the treatment of high-level nuclear wastes (Opperman et al., 2011).

Recently, the detection of parabens was performed by replacing salt with an ionic liquid in an aqueous two-phase system method. In this method, parabens are mixed with ionic liquid, salt, and deionized water. The mixture then separates into two aqueous layers: an ionic liquid-rich phase and an aqueous-rich phase. The upper layer is then analyzed by high-performance liquid chromatography (HPLC), and the data obtained will show the peak area of parabens detected in that particular sample. Percentage of recovery is calculated based on the peak area. Hence, the concentration of parabens can be determined using a plotted calibration curve.

During testing, the ionic liquid method recovered 80 to 90% of the parabens (Noorashikin et al., 2014). The method also improved selectivity by reducing the matrix effect, which can cause a reduction in the detection level. It is thus feasible to detect parabens in environmental water samples using this method.

**Further reading**


The spotlight is on AOCS Platinum and Silver Corporate Members

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ADM
BUNGE

Louis Dreyfus Commodities

Monsanto

Gold

Canadian Grain Commission
Commission canadienne des grains

CLARIANT

Kao

BIO DIESEL BOARD
oil:dri

Fluids purification

Silver

AB Enzymes
AGP Ag Processing Inc

ANDERSON INTERNATIONAL CORP
BASF
BERGESON & CAMPBELL PC

BIOGRA
Colonial Chemical
Corbion

CROLL REYNOLDS
Process Vacuum | Air Pollution Control

Dow
Dow AgroSciences

FRENCH
HERSCY

Huntman

Intertek

PQ Corporation

Process Plus

P&G

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(As of April 30, 2017) (b) * ADM is a registered trademark of Archer-Daniels-Midland Company
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The spotlight is on AOCS Platinum and Silver Corporate Members

(As of April 30, 2017.)

* ADM is a registered trademark of Archer-Daniels-Midland Company
In 2011, the European Commission decided to develop Europe into the first bio-based economy globally. To set the basis for this development, the Commission issues several mandates to European Standardization bodies, such as the European Commission for Standardization (CEN), for the development of horizontal standards on bio-based products in general, and vertical standards for selected product groups, such as bio-lubricants, bio-based solvents, and bio-based surfactants [1].

CEN installed the new technical committee CEN/TC-411 “Bio-based products” to create new general standards for providing a common basis with respect to common terminology, bio-based content determination, sustainability aspects, life cycle assessment LCA, and declaration/communication tools for B2B and B2C [2].

Based on these new horizontal standards, a new working group within the Technical Committee, CEN/TC-276 “Surface active agents,” took on the task of standardizing bio-based surfactants in cooperation with the ERASM Biosurfactant Taskforce (ERASM = Environmental Risk ASessment and Management, www.erasm.org).

STANDARDIZATION
Bio-based raw materials have been used for millennia in the manufacture of surfactants. The first surfactant made by humans—soap—was already completely bio-based. With the advent of modern surfactants in the early 20th Century, petrochemical-based raw materials also gained interest. They
offered the opportunity to tune the surfactant properties, in a broader sense, to their various applications.

The last decades have seen the emergence of new bio-based raw materials for surfactants. Some of the reasons for the increased interest lie in the potential benefits bio-based products offer with respect to offsetting the depletion of fossil resources and climate change.

Surfactants consist of at least one hydrophobic and one hydrophilic part. The source of these parts can be either fossil-based or bio-based (renewable).

Traditionally, triglycerides from various oil plants are used as renewable sources for the hydrophobic part. Suitable plants include oil palms, coconut palms, sunflowers, rapeseeds, or soy beans, among others. The oils taken from these plants are further chemically processed to get fatty acids by saponification or to get fatty alcohols by methanolysis of the triglycerides and consecutive hydrogenation of the fatty methyl ester. Further sources of triglycerides may be algal oils or fermentation products.

If fossil resources are used for the hydrophobic part, there are myriad pathways to process either crude oil or natural gas to build the hydrophobic building block. These include such pathways as Fischer-Tropsch synthesis, oxo process, olefin oligomerization, or Friedel-Crafts alkylation.

Generally, the carbon number of the hydrophobic part of a surfactant ranges from 4 to 24 carbon atoms, whereas the majority has a carbon number between 10 and 15 carbon atoms.

The hydrophilic part may also be derived from different sources—either organic from renewable or fossil sources or inorganic from minerals. Some examples of hydrophilic components and their origin can be seen in Table 1.

**TABLE 1. Examples of hydrophilic components of surfactants and their origin**

<table>
<thead>
<tr>
<th>Hydrophilic source</th>
<th>Type of material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renewable</td>
<td>Sugar, starch</td>
</tr>
<tr>
<td>Fossil</td>
<td>EO, chloroacetic acid</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Sulphate, sulfonate, phosphate</td>
</tr>
</tbody>
</table>

Determining biomass in a bio-based product (surfactant) is crucial for a standard. There are two ways to do this: by analytical measurement, or by certification along the value chain. The analytical radiocarbon method (ASTM D6866-12, EN 16640 and others) relies on the content of the carbon isotope $^{14}C$, which allows for a clear distinction between carbon-based substances in present living organisms and carbon-based substances from fossil sources. The suitability of this method for bio-based surfactants was being investigated at the time this article went to press. Certification from such organizations as RSPO (Round Table of Sustainable Palm oil), ISCC (International Sustainability & Carbon Certification), and others can also be used.

Based on the biogenic carbon content as a measure for biomass content, the CEN working group has established a classification for bio-based surfactants (Table 2). The rationale behind this classification is based on:

- **the consistency between threshold levels and nomenclature,**
- **the self-explanatory nomenclature,**
- **the possibility to establish a market monitoring,** and
- **the potential use in ecolabel criteria, such as EU flower.**

Examples of wholly bio-based surfactants include alkyl polyglucosides, sorbitan esters, and alcohol sulfates. Examples of majority bio-based surfactants include most alkyl ethersulfates, betaines, and esterquats.

In addition to determining the origin of raw materials, it is also necessary to assess the impact on sustainability and potential end-of-life options of bio-based surfactants.

**TABLE 2. Bio-based surfactant classes (taken from CEN/TS 17035)**

<table>
<thead>
<tr>
<th>Surfactant class</th>
<th>Bio-based carbon content X% (m/m)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholly bio-based surfactant</td>
<td>≥ 95</td>
<td>Applicable for surfactants, where all raw material can be considered as bio-based</td>
</tr>
<tr>
<td>Majority bio-based surfactant</td>
<td>95 ≥ X &gt; 50</td>
<td>Applicable for surfactants, where the majority of the raw material is bio-based</td>
</tr>
<tr>
<td>Minority bio-based surfactant</td>
<td>50 ≥ X ≥ 5</td>
<td>Applicable for surfactants, where the minor part of the raw material is bio-based</td>
</tr>
<tr>
<td>Non bio-based surfactant</td>
<td>x &lt; 5</td>
<td>Applicable for surfactants where no raw material is bio-based</td>
</tr>
</tbody>
</table>
Table 3 describes the origin of raw materials and their impact on end-of-life. It shows that there is no straight correlation between the origin and the biodegradability of surfactants under OECD 301 test conditions. It is just one of many aspects that should be considered. Performance of the surfactants and their contribution to the application for which they are used depend not on the origin, but on the structure of any surfactant. This is the dominating reason why there should be no reduction in sustainability for new bio-based surfactants versus surfactants that are already in use, regardless of whether they are fossil-based or bio-based.

MARKET SITUATION IN EUROPE
Surfactants are produced by multinational companies as well as SME’s. Figure 1 shows the use of bio-based and non bio-based surfactants in EU (+ Norway, Iceland, and Switzerland) according to the classification in Table 2 in 2015. The split is close to being equal. Partitioning the bio-based into the proposed bio-based surfactant classes shows further details about their use.

WAY FORWARD
The task of standardizing bio-based surfactants in Europe is not finished yet. The analytical project to evaluate the radiocarbon method for determining the biomass of bio-based surfactants is still under way, as is the upgrading of CEN/TS 17035 to a European norm. Once plans for the new European norm are in place, the working group will issue a Technical Report explaining the background and rationales. The goal is to finish all drafts by end of 2017.

Juergen G. Tropsch started his career by working as a research chemist at BASF in 1990. In 1998, he switched to development and application technology for surfactants. He was promoted to Senior Expert for Surfactants in 2007, and in 2009 received the Innovation Award of BASF for the development of a new class of nonionic surfactants. Since 2013, Tropsch has headed the working group 3 for bio-surfactants within CEN/TC-276. He can be contacted at juergen.tropsch@basf.com.

Further reading
[2] For further detail see EN 16575, EN 16640, EN 16785-1, EN 16785-2, EN 16751, EN 16760, EN 16848, and prEN 16935

TABLE 3. Relation between raw material origin and biodegradability accord. OECD 301 (CTAB = cetyltrimethylammonium bromide)

<table>
<thead>
<tr>
<th>Product group</th>
<th>Bio-based raw materials</th>
<th>Biodegradability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol ethoxylates</td>
<td>mixed/no</td>
<td>yes</td>
</tr>
<tr>
<td>Alcohol alkoxylates</td>
<td>mixed/no</td>
<td>yes/no</td>
</tr>
<tr>
<td>Alkylphenol ethoxylates</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>EO/PO blockpolymers</td>
<td>no</td>
<td>yes/no</td>
</tr>
<tr>
<td>Alcohol sulfates</td>
<td>yes/no</td>
<td>yes</td>
</tr>
<tr>
<td>Alcohol ether sulfates</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Alkyl polyglucosides</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Linear alkylbenzenesulfonates</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Quaternary ammonium compounds (CTAB)</td>
<td>mixed</td>
<td>no</td>
</tr>
<tr>
<td>Quaternary ammonium compounds (Esterquats)</td>
<td>mixed</td>
<td>yes</td>
</tr>
<tr>
<td>Betaines</td>
<td>mixed</td>
<td>yes</td>
</tr>
</tbody>
</table>
Fruit seeds are known to be promising sources of oils rich in carotenoids, phenolic compounds, tocopherols, and phytosterols. The citrus industry has great potential for growth, and is one of the most competitive agricultural sectors. Oranges are extensively processed to obtain natural juices, pulps, candies, and extracts. However, wastes generated by this processing represent approximately 50% of the fruits (Hernández-Montoya et al., 2009). Better use of these by-products would not only minimize environmental impacts, but would add value by turning material that would otherwise be disposed of into useful co-products for application in food, pharmaceuticals, and cosmetics (Schieber et al., 2001). For these reasons, our group set out to characterize the oils extracted from the seeds in orange waste, and determine their antioxidant activities.

OBTAINING THE OILS

The orange varieties most commonly used to produce juice in the state of São Paulo, Brazil, are Hamlin, Natal, Pera-rio, and Valencia. They belong to the species *Citrus sinensis* (L.) Osbeck, and are known for their sweet taste. The flowers and fruits of these oranges are smaller than those of other varieties, and they also have a thinner peel and pith (Koller, 2006).

Three batches of waste from each variety were acquired from the 2010 harvest in Indústria Suco Cítrico Cutrale in Uchôa, São Paulo, Brazil. The seeds were manually separated from the peels and pulp, and dried on trays for approximately 96 h, at room temperature, to reduce moisture (< 10%). The batches of seeds from each variety were then weighed, homogenized, packed in rigid plastic polypropylene packaging, sealed with screw caps, and properly labeled for further analysis. The seeds were later pressed in a vegetable oil extractor, at room temperature, with an initial rotation of 25 Hz and a final rotation of approximately 60 Hz. The extracted oils were placed in amber glass bottles with nitrogen gas and stored at -18 °C for further analysis.
ANALYZING THE OILS

Total carotenoids were determined by spectrophotometry according to the method described by Rodriguez-Amaya (1999). The quantification was performed in a UV-vis spectrophotometer with a wavelength interval from 300 nm to 550 nm. Quantification was measured by absorption wavelength of maximum absorbance and absorptivity value of 2592 in petroleum ether, expressed as milligrams of β-carotene per kilogram.

The total phenolic compounds were extracted from the oil samples using the procedure described by Parry et al. (2005). The compounds were then quantified by spectrophotometry, using Folin-Ciocalteu reagent (Singleton and Rossi, 1965), through calibration curve with gallic acid as standard. The levels of total phenolic compounds in oils were expressed as grams of gallic acid equivalents (GAE) per kilogram of oil.

The determination of tocopherols was performed according to AOCS Ce 8-89 (2009) using high-performance liquid chromatography (HPLC) with a fluorescence detector. The quantification was conducted by external standardization, and the values were determined based on peak areas and expressed in values of each isomer, separately, in milligrams per kilogram.

The contents of phytosterols extracted from the seeds were determined by gas chromatography, with previous saponification of the samples. Saponification was performed according to the method published by Duchateau et al. (2002). Phytosterols were determined by making adaptations to AOCS Ch 6-91 (2009). The analyses were carried out in a gas chromatograph, equipped with a flame-ionization detector, split-splitless injector, and an autosampler. The compounds were separated in a fused silica capillary column. The programming of the temperature column was initiated at 100 °C, for 2 min, heated at 15 °C/min until 260 °C, and kept isothermal for 35 min. The temperatures used in the injector and in the detector were 280 and 320 °C, respectively. The carrier gas was hydrogen with a linear velocity of 40 ml/min. Samples of 1.0 μL were injected with split ratio of 1:50. Sterols (cholesterol, campesterol, stigmasterol, β-sitosterol, and stigmastanol) were identified by comparison with the retention time of pure standards (Supelco, Bellefonte, Pennsylvania, USA), and analyzed under the same conditions of the samples. The quantification of each isomer was performed by internal standardization (5α-cholestan-3β-ol), based on the peak areas.

The antioxidant activity of the oils was determined according to the method described by Kalantzakis et al. (2006). This method consists of evaluating the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). To determine the antioxidant activity of the oils, 1 g of oil was diluted with 10 ml of ethyl acetate. From this solution, 1 ml was added to 4 ml of a DPPH• solution, in 10⁻⁴ mol/l ethyl acetate, and was vigorously shaken in vortex, for 10 sec. After 30 min in the dark, the mixture absorbance was measured at 517 nm. A control sample (without oil) was prepared and the absorbance was equally measured. The levels of absorbance obtained were converted to percentage of antioxidant activity (AA).

The efficient concentration (EC), defined as the sufficient concentration to obtain 50% of the maximum effect estimated in 100% (expressed in kilograms of oil per kilogram of DPPH•), was graphically determined. To do so, oil samples were diluted with ethyl acetate in concentrations of 10, 25, 50, 75, and 100
mg/ml. Measurements of the absorbance of reaction mixtures (1 ml of solution sample and 4 ml of DPPH• in ethyl acetate) were performed at 517 nm at 0 and 30 min. The antiradical efficiency (AE) of oils was determined according to Equation 1 (Brand-Williams et al., 1995)

\[ AE = \frac{1}{EC_{50}} \]  

The results obtained from analytical determinations, in triplicate, were submitted to analysis of variance, and differences between means were tested at 5% probability, by Tukey test (Gacula Jr. et al., 2009).

**CAROTENOIDS, PHENOLIC COMPOUNDS, AND TOCOPHEROLS**

The levels of total carotenoids, total phenolic compounds, and tocopherols in the oils of Hamlin, Natal, Pera-rio, and Valencia seeds are shown in Table 1.

**TABLE 1.** Total carotenoids, total phenolic compounds, and α-tocopherol of oils extracted from orange seeds

<table>
<thead>
<tr>
<th>Varieties</th>
<th>TC (mg/kg)</th>
<th>TPC (g/kg)</th>
<th>α-tocopherol (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamlin</td>
<td>11.64 ± 0.54c</td>
<td>3.79 ± 0.03a</td>
<td>135.50 ± 0.29b</td>
</tr>
<tr>
<td>Natal</td>
<td>18.47 ± 0.78b</td>
<td>4.80 ± 0.01b</td>
<td>134.07 ± 0.21c</td>
</tr>
<tr>
<td>Pera-rio</td>
<td>26.69 ± 0.27a</td>
<td>4.91 ± 0.01a</td>
<td>137.43 ± 0.16a</td>
</tr>
<tr>
<td>Valencia</td>
<td>19.24 ± 0.93b</td>
<td>4.21 ± 0.04c</td>
<td>135.63 ± 0.49b</td>
</tr>
</tbody>
</table>

The results represent the mean ± standard deviation of the analyses performed in triplicate. Means followed by the same letter in the lines do not differ by Tukey test (p < 0.05). TC (total carotenoids) are expressed as β-carotene; TPC (total phenolic compounds) are expressed as GAE equivalents.

The oil of Hamlin orange seeds presented the lowest amount of total carotenoids (11.64 mg/kg, expressed as β-carotene) and the oil of Pera-rio orange presented the highest amount, 26.69 mg/kg. The oils of Natal and Valencia orange seeds did not differ significantly by Tukey test (p > 0.05), with values of 18.47 mg/kg and 19.24 milligrams of β-carotene per kilogram, respectively.

Malacrida et al. (2012) evaluated Pera-rio orange seed oils, and found levels of 0.13 mg/kg and 0.19 mg/kg of lutein and β-carotene, respectively. The corn germ oil presented 5 mg/kg of total carotenoids (Moreau et al. 2007). The above mentioned values are lower than the ones presented by orange seed oils in the present study.

Xu et al. (2008) found total carotenoids concentrations of 0.08, 2.92, and 0.72 mg/ml (expressed as β-carotene) in lemon, ponkan, and Hamlin orange juices, respectively.

The content of total carotenoids in oils is affected by the maturation stage of the fruits, and by their extraction and storage conditions. Oils extracted from ripe fruits may present higher amounts of carotenoid pigments, while those obtained from partially ripe fruits have higher chlorophyll concentration (Ramadan and Moser, 2003). All oils showed important levels of total phenolic compounds: The highest content was present in the oils of Pera-rio orange seeds, followed by those of Natal, Valencia, and Hamlin.

Malacrida et al. (2012) studied the phytochemicals and antioxidant activity of citrus seed oils. The level of total phenolic compounds of Pera-rio orange seed oil was determined by using the Folin-Ciocalteu reagent under the same analytical conditions, and the results were expressed as gallic acid equivalents per kilogram of oil. According to the results, this oil obtained 1.15 g/kg of total phenolic compounds. The levels of phenolic compounds in the orange seed oils from this study were higher than those found in the oils analyzed in the mentioned study.

When analyzing lemon, ponkan, and Hamlin orange juices, Xu et al. (2008) found concentrations of total phenolic compounds of 751.82, 830.32, and 1499.71 g/kg, respectively using the Folin-Ciocalteu reagent.

In soybean, sunflower, corn, canola, and rice oils extracted by cold extraction, the quantities of total phenolic compounds ranged from 126 g/kg to 148 g/kg, in caffeic acid equivalents (Siger et al., 2008), and in olive oil from 0.16 mg/kg to 0.40 g/kg, in gallic acid equivalents (Nakbi et al., 2010).

The quantification of these substances is influenced by the nature of the compound, the method of extraction employed, as well as by the presence of interfering elements, such as waxes, terpenes, and chlorophyll. A satisfactory method for extraction of phenolics present in the samples has not been developed yet.

As seen in Table 1, only α-tocopherol was detected in the oils extracted from seeds of all orange varieties. Since α-to-

**TABLE 2.** Amount of phytosterols of oils extracted from orange seeds

<table>
<thead>
<tr>
<th>Phytosterols (mg/kg)</th>
<th>Varieties</th>
<th>Per-rio</th>
<th>Valencia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hamlin</td>
<td>Natal</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>23.0 ± 0.0c</td>
<td>36.9 ± 0.0a</td>
<td>15.1 ± 0.0d</td>
</tr>
<tr>
<td>Campesterol</td>
<td>60.2 ± 0.0c</td>
<td>81.7 ± 0.0a</td>
<td>76.5 ± 0.0b</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>1,205.3 ± 0.0b</td>
<td>1,215.4 ± 0.0a</td>
<td>1,203.1 ± 0.0c</td>
</tr>
<tr>
<td>Totals</td>
<td>1,288.5</td>
<td>1,334.0</td>
<td>1,294.7</td>
</tr>
</tbody>
</table>

The results represent the mean ± standard deviation of the analyses performed in triplicate. Means followed by the same letter in the lines do not differ by Tukey test (p < 0.05). Only cholesterol, campesterol, and β-sitosterol were detected in the samples analyzed.
copherol presents higher biological activity than vitamin E, the oils analyzed may present vitamin activity.

Anwar et al. (2008) determined the composition of tocopherols in oils extracted from citrus species seeds (C. paradisi, C. sinensis, C. reticulata) and also obtained α-tocopherol as the main tocopherol, 380, 220, and 557.82 mg/kg, respectively. The amounts of total tocopherols in orange seed oils were reported to be higher than those in babaçu oil (60–130 mg/kg), similar to those in palm stearin (100–700 mg/kg), and lower than those in soybean oil (600–3370 mg/kg) (Codex Alimentarius Commission, 2009).

**PHYTOSTEROLS**

Phytosterols are of great interest due to their antioxidant activity and their impact on health. They are the main components of the unsaponifiable matter in oils, and analyzing sterols provides information about the quality of the oil. Phytosterols are among the constituents of the cell wall in vegetables. Because their molecular structure is similar to that of cholesterol, they reduce the absorption of cholesterol by the intestine. Purified phytosterols and phytostanols have been added to foods to increase hypocholesterolemic activity. A daily intake of 1.6–2.0 g/day of phytosterols and phytostanols can reduce intestinal absorption of cholesterol by up to 30%, and lower the level of low-density lipoprotein (LDL) cholesterol in blood by 8–10% (Marangoni and Poli, 2010). The amount of phytosterols in the orange seed oils we studied are displayed in Table 2.

The oil of Natal orange seeds presented higher amounts of the three phytosterol isomers: β-sitosterol, campesterol, and cholesterol, as well as the highest levels of total phytosterols.

When studying bitter melon, kalahari melon, kenaf, pumpkins, and rosette oils, Nyam et al. (2009) found levels of total phytosterols of 4,643, 6,416.90, 3,675.60, 2,740, and 7,575.60 mg/kg, respectively. Nehdi et al. (2010), reported 3,360.70 mg/kg of total phytosterols in Phoenix canariensis seed oils. However, orange seed oils contain lower amounts of these compounds compared to the percentage of total phytosterols in palm oil (270–800 mg/kg) (Codex Alimentarius Commission, 2009).

Arena et al. (2007) obtained 100.40 mg/kg of total phytosterols for pistachio seed oils. Cheikh-Rouhou et al. (2008) found levels of total phytosterols in Nigella sativa and Pinus halepensis seed oils of 281 mg/kg and 735 mg/kg, respectively. Such values are lower than those of orange seed oils.

It is important to note that the content of phytosterols detected in samples may vary depending on the chromatographic conditions employed, such as the programming of the temperature column, the temperatures used in the injector and in the detector, the carrier gas speed, among other variables.

**ANTIOXIDANT ACTIVITY**

The antioxidant activity (%), the concentration of the oil necessary to reduce the free radicals (EC50) in 50%, and the antiradical efficiency, are presented in Table 3.

### Table 3. Antioxidant activity, EC50 and antiradical efficiency of oils extracted from orange seeds

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Antioxidant activity (%)</th>
<th>EC50 (kg/kg)</th>
<th>Antiradical efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamlin</td>
<td>58.9 ± 0.7a</td>
<td>37.19</td>
<td>2.69 × 10⁻²</td>
</tr>
<tr>
<td>Natal</td>
<td>56.0 ± 0.5b</td>
<td>37.25</td>
<td>2.68 × 10⁻²</td>
</tr>
<tr>
<td>Pera-rio</td>
<td>70.2 ± 0.4c</td>
<td>35.08</td>
<td>2.79 × 10⁻²</td>
</tr>
<tr>
<td>Valencia</td>
<td>59.9 ± 1.0d</td>
<td>38.31</td>
<td>2.61 × 10⁻²</td>
</tr>
</tbody>
</table>

The results represent the mean ± standard deviation of the analyses performed in triplicate. Means followed by the same letter in the column do not differ by Tukey test (p < 0.05). EC50 is defined as the sufficient concentration to obtain 50% of the maximum effect estimated in 100% (expressed in kilograms of oil per kilogram of DPPH•).

All the orange seed oils showed DPPH• radical scavenging activity. However, the oil of Pera-rio orange seeds was the most effective, presenting antioxidant activity of 70.2%, while the oils of Pera-rio orange seeds presented the highest value for antiradical efficiency (2.79), which was determined by using EC50 value. Antioxidant activity was significantly correlated with α-tocopherol levels (r = 0.81), indicating that the oils with higher concentrations of α-tocopherol presented higher radical scavenging activity.

In evaluating the antioxidant activity of citrus oils, Malacrida et al. (2012) found the oil obtained from Pera-rio orange seeds to be highest in antioxidant activity (54.2%). The orange seed oil presented higher DPPH• scavenging activity among the analyzed oils, reaching 47.6% of the DPPH• in the reaction mixture. The orange oil also showed the best EC50 (10.75 g oil/g DPPH•) and antiradical efficiency (9.30 × 10⁻¹). The levels of antioxidant activity and EC50 in the orange seed oils analyzed by Malacrida et al. were higher than in the oils analyzed in our analysis.

All oils analyzed in this study presented considerable amounts of carotenoids, phenolic compounds, α-tocopherol, and phytosterols. They also demonstrated free radical scavenging capacity, with antiradical efficiency following a decreasing order: Pera-rio > Hamlin = Natal > Valencia. Such results indicate that specialty oils derived from the wastes of orange processing could be a potential source of healthful bioactive compounds and antioxidants.

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Sharing goodness around the world

Utkarsh Shah, a senior research scientist at The Hershey Company, has a lot of expertise and experience to share. As a food scientist who specializes in fats and oils, Shah is responsible for developing the technologies and facilities for new product development at Hershey, where he often participates in long-term research projects. He was instrumental in launching recent snack products and worked on Hershey’s Spreads, among many other products.

Shah recently put these skills to good use as a volunteer for a Partners in Food Solutions (PFS)-sponsored project in which he worked with a company in Ghana to convert a manufacturing plant to edible oil production. Shah served as a quality expert for the conversion process, building up quality programs for understanding and measuring the oil quality produced at the plant. After four months of work, Shah’s volunteer team finished compiling all of its recommendations to be shared with the company. Shah’s recommendations included installing a small quality lab for analyzing the oils processed at its facility. He provided specifications for the lab, along with several methods for analyzing the oil, and quality criteria to ensure oil safety and edibility. Once implemented, the company will have a new product that promises to increase business and revenue.

While Shah clearly put a lot of his own knowledge and expertise into the project, he says he learned a lot from it, too. For one, it gave him a broader outlook. “Working with a very small company in Ghana helped me to realize that things are not as simple as they seem—and that there are a lot of things to consider when you start a plant, down to the smallest details,” he says. “Resources are limited, and finding funding sources is difficult for these small companies. This slows down the implementation process.” Shah also benefitted from communicating with a different culture, across a long distance.

With respect to other challenges, Shah laughingly admits that he is not a morning person, so he had to cope with early morning calls to accommodate the time difference when working with people across the ocean. Another challenge, one of the biggest, was communication. Shah was always mindful of background information that was missing due to working on a project from so far away. He says he asked a million questions to make sure he understood the situation as completely as possible. “That was just part of the challenge, to figure out what was going on, and how to make the most of the limited communication we had,” he says.

Another challenge was limited time. “Being a volunteer, you can’t spend 20 hours a week. It’s just two or three hours a week, so the question becomes, ‘How can you make the most impact?’ That was the biggest challenge—you really want to help, but you can only give so much time, because you have responsibilities with your regular job. But I think we managed well, and did a good job.”

Despite the challenges, Shah always found the project interesting and motivating. “There is a sense of satisfaction in working for a cause that really needs the help,” he says. “There is a big problem with food security in the world. The challenge is to provide the growing population with safe, affordable, nutritious food, sustainably. Being a food scientist, the more I can work on this issue and make a tangible contribution to society, the more satisfaction it gives, and motivates me to keep working on these projects.”

Shah has a lot to say to others considering volunteering with PFS. “What we have learned so far in our career of being a food scientist or professional is great, but in order to really build that satisfaction—one way of doing philanthropy work is to give money, but here we are, experts in our field, and by volunteering for a good cause and sharing our knowledge, it really gives a deep satisfaction. That satisfaction translates to the people we’re helping and also into our career. I would encourage people to look at volunteering with PFS. It’s not difficult. If you have the expertise and are part of a good team, you’ll have satisfaction. And it’s just a good thing to do, to help another part of the world. Moreover, it is an honor to build on Mr. Milton Hershey’s legacy to spread goodness around the world by sharing my expertise for such a special social cause and making a difference.”

Shah has since expanded his PFS role, serving as client lead for his next project, which is also in Ghana. The new client, Home Foods, processes and packages various types of ready-to-eat foods, ethnic foods, and seasoning products for export. Founded by a graduate of Kwame Nkrumah University of Science & Technology and the Harvard Business School, the company is 100% Ghanaian-owned and sources its agricultural materials from more than 6,000 cooperative women growers and suppliers. Shah’s project will focus on a factory recently acquired by the growing company and on building a human resources and business strategy as that company creates new operations for ready-to-eat foods.

He says it was the work with PFS itself that kept him engaged, but what keeps him coming back is the satisfaction of using his expertise for the betterment of the world.

Utkarsh Shah is a member of Inform’s editorial advisory committee and the AOCS Young Professional Common Interest Group. This article about his work in Ghana originally appeared on the Partners in Food Solutions (PFS) website (http://www.partnersinfoodsolution.com/about), and has been modified and republished with permission.
The continuing concern with how to deal with oil spills is leading to research exploring new approaches. A Janus fabric coated with a single multifunctional polymer was found to be very effective in removing oil from emulsions [1]. Depending upon the environment, a specific functional group in the polymer rises to the top of the coating to first break the emulsion and then isolate the oil droplets.

One strategy needing further exploration is finding an environmentally friendly method for cleaning up oil spills. This is a particularly important issue in a remote location such as the Arctic Ocean. George Bonheyo, senior research scientist at the Pacific Northwest National Laboratory (PNNL) and professor of bioengineering at the Gene and Linda Voiland School of Chemical Engineering and Bioengineering at Washington State University in Pullman, Washington, USA, says, “With the ice retreating in the Arctic Ocean, further oil exploration and production will be occurring that will require better oil spill response methods that are effective in the icy, cold waters present in this region.”

In looking to find a solution, Bonheyo initially established a renewable strategy and wanted a material that readily absorbed oil. The direction that Bonheyo took was to evaluate the potential for using sawdust. He says, “We found that sawdust is a good carrier for microbes in bioremediation applications. Sawdust stabilizes the microbes and absorbs their toxins.”

Sawdust consists of cellulose, hemicellulose, and lignin. While this material is not water soluble, it needs to be made more hydrophobic and less dense. The latter issue is important because the oil spill agent must float on seawater to be effective.

A new approach has been developed to convert sawdust into a material suitable for absorbing oil spills.

MODIFIED SAWDUST
Bonheyo and his colleagues have modified sawdust to make the material more hydrophobic and less dense so that it can absorb oil and remain on the surface of salt water for at least four months. He says, “We determined that sawdust can be modified by reacting the material with vegetable fatty acids at elevated temperatures.”

Bonheyo’s reasoning is that oil has a broad molecular distribution, so a sawdust modified with a broad range of fatty acids is best able to absorb it. He says, “The inspiration for making the sawdust hydrophobic stems from work we did
to develop superhydrobic coatings that act as anti-biofouling surfaces.”

The fatty acids are incorporated into sawdust by reacting with the hydroxyl groups present in the lignin. The resulting material is less dense than seawater and is able to hold oil on the surface of the water for a long period of time. Thermogravimetric analysis was used to determine the degree of esterification in the modified sawdust.

The efficacy of the modified sawdust was ascertained by adding the material to seawater in a customized shipping container that can be cooled down to as low as -15°C (5°F). Testing at that temperature is particularly useful because ice slush can form on the surface of the water. Bonheyo indicated that the hydrophobic nature of the modified sawdust enabled the material to prevent ice from forming on its surface.

Several tests were run to assess the performance of the modified sawdust. Bonheyo says, “We evaluated the buoyancy of the material and found that it does not sink into the water. No emulsion is formed either with seawater or with distilled water.”

Modified sawdust also was smeared on a glass slide to determine the material’s contact angle. Bonheyo says, “We found the contact angle to be greater than 115 degrees.”

Oil absorption capacity showed that the modified sawdust can absorb up to five times its weight in oil. Burn efficiency also was evaluated because this is a rapid and efficient way to dispose of the oil in the harsh environment of the Arctic Ocean before it can cause greater harm. Bonheyo says, “An efficient burn will remove 90% of the oil, leaving a small amount of tarry residue that is enriched in large poly-aromatic hydrocarbons (e.g., asphaltene) that can be difficult to remove.” Burn tests were performed at PNNL’s Marine Sciences Laboratory in Sequim, Washington, USA, and at the US Coast Guard and Naval Research Laboratory’s Joint Maritime Test Facility (JTMF) in Mobile, Alabama, USA.

The modified sawdust also can be used on land in an industrial setting. Bonheyo says, “The most effective way to use the modified sawdust may be to add bacteria and fungus to the material to facilitate the decomposition of the oil. Water should be added to accelerate microbial activity. Byproducts of microbe metabolism are readily absorbed by the modified sawdust.”

Bonheyo recommends the use of aerobic microbes and believes the modified sawdust can be safely landfilled. The researchers will continue analyzing the properties of the modified sawdust and are hoping that the US government will be interested in evaluating the material in the future.

The research was funded by the US Bureau of Safety and Environmental Enforcement.

Additional information can be found at www.pnnl.gov/news/release.aspx?id=4333, or by contacting Bonheyo at george.bonheyo@pnnl.gov.

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Further reading

More than 20 years ago, the US Food and Drug Administration (FDA) implemented conditions for use of the term “healthy” on food labels. To qualify as healthy, a packaged food must be low in total fat, saturated fat, and cholesterol; relatively low in sodium; and contain at least 10% of the daily value for vitamin A, vitamin C, calcium, iron, protein, or fiber. In those days, fat was considered the number one enemy of a healthy diet, whereas sugar was barely on the nutritional radar. Since then, nutrition science has evolved dramatically, and increasing evidence indicates that cutting sugar and refined carbohydrates is more effective than a low-fat diet for the prevention of obesity and cardiovascular disease.

“The current FDA definition of healthy is notably out of alignment with the recommendations of the 2015 Dietary Guidelines for Americans,” Melissa Musiker, director of APCO Worldwide, told Food Navigator. “It also doesn’t align with what most reasonable people, health experts, or nutritionists would consider to be sound nutritional advice.” For example, sugary cereals, fat-free puddings, and low-fat toaster pastries could be labeled “healthy” under the FDA’s current rules, whereas almonds, salmon, or avocados could not because of their high fat content.

Two years ago, a highly publicized case caused the FDA to admit that its definition of “healthy,” particularly with regard to fat, is outdated and needs revision. In March 2015, the FDA sent a warning letter to the makers of KIND fruit and nut bars saying that some of the bars “did not meet the requirements for use of the nutrient content claim ‘healthy’” because they contained too much fat per serving. The action left KIND vulnerable to a slew of class-action lawsuits contending that KIND violated consumer protection laws. In response, KIND filed a citizen’s petition with the FDA requesting that the organization update its regulations on the use of the term “healthy” on food labels.

In a rare about-face, the FDA agreed with the KIND executives’ position. “We do not object to the specific statement that you would like to place on your bar wrappers, on the condition that there will be no other nutrition-related statement, such as express or implied nutrient content claims, on the same panel of the label,” wrote Susan Mayne, Director of the Center for Food Safety and Applied Nutrition at the FDA, in a letter dated April 22, 2016. “We agree with you that our regulations concerning nutrient content claims are due for a reevaluation in light of evolving nutrition research.”

Moreover, the FDA issued a guidance document, effective immediately, stating that the FDA will not enforce the current regulatory requirements for “healthy” labeling in products that 1) are not low in total fat, but contain predominantly mono- or polyunsaturated fats, or 2) contain at least 10% of the daily value of potassium or vitamin D per serving.

To help update the criteria for “healthy” label claims, the FDA solicited input from stakeholders and consumers via a written and electronic public comment process, which closed April 26, 2017. In addition, a public meeting, held on March 9, 2017, invited stakeholders in the food and beverage industry to discuss such topics as consumer perceptions of “healthy,” and whether the term should be defined on the basis of specific nutrients or of food groups. Now that the public comment period has closed, the FDA will review the comments and revise its definition of “healthy,” although no specific timeline has been proposed.

In the meantime, speculation is rife on what the new definition of “healthy” will encompass. Currently, to make the “healthy” claim, packaged foods must contain no more than 3 g total fat and 1 g saturated fat per serving. The FDA may decide to increase the allowable fat content, or totally eliminate restrictions on fat. However, the organization will likely retain a limit on saturated fat because these fats are still considered less healthful than mono- or polyunsaturated fats. The restriction on cholesterol in healthy foods may be removed, given that the 2015 Dietary Guidelines Advisory Committee concluded that cholesterol is no lon-
A limit on added sugars will likely be added to the new definition of “healthy,” in accordance with the 2015 Dietary Guidelines for Americans and the FDA’s recently revised Nutrition Facts label. And the requirements for vitamins A and C are likely to be traded for stipulations on potassium and vitamin D. When the FDA defined “healthy” in 1993, many Americans were deficient in vitamins A and C, but now potassium and vitamin D are of greater public health concern.

Meanwhile, KIND company CEO Daniel Lubetzky has not rested on his laurels. He recently pledged $25 million USD to establish a public advocacy organization called Feed the Truth, which will combat “Big Food’s” influence on FDA regulations and the public’s perceptions of “healthy.” Lubetzky contends that food companies have funded studies that downplay the role of sugar and salt in disease while vilifying fat, which has, in turn, affected government nutrition policies. To avoid a conflict of interest, Lubetzky plans to remove himself completely from the activities and leadership of Feed the Truth, which will be governed by an independent Board of Directors. The activities of the new organization could include grants to support investigative journalism, consumer education campaigns, and educational briefings for policymakers. In a news release dated February 15, 2017, Lubetzky acknowledges “Big Sugar’s” influence on shaping nutrition policy, but says “it doesn’t mean that ‘fat’ or ‘protein’ should now be evangelized. Feed the Truth’s independent Board of Directors, once established, will seek to ensure consumers have access to unbiased nutrition information.”

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


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EU chemicals regulations “failing” consumers, member states say

Lack of information and unclear labelling are leaving consumers in the dark when it comes to hazardous substances in products, EU member states say.

And most consumers do not have the knowledge to “discriminate effectively” between different categories of warnings on product labels, the UK Health and Safety Executive (HSE) says.

Alongside other member states, industry bodies and NGOs, the UK HSE has submitted comments to the European Commission’s consultation on the regulatory fitness of chemicals legislation, excluding REACH.

In its comments, Germany’s environment agency (UBA) gives an example in the classification “corrosive,” as regulated under the Detergents Regulation. This, it says, does not allow any differentiation between “slightly” and “strongly” corrosive.

UBA says the Detergents Regulation also does not ensure that the obligation of Article 11 (2)—which says certain information must appear in legible and visible characters—is “fulfilled directly” on large containers used for refilling of detergents. As such, necessary amendments should be made to Article 11 so that consumers are not misled, it adds.

For cosmetics products, while the list of ingredients must be indicated on the packaging, information is missing on which chemicals are hazardous, the German agency says.
Other mixtures, such as paints and varnishes, must bear precautionary statements to ensure appropriate handling by consumers, it says, and the same should be applied to cosmetics. This would help consumers make an informed choice when they buy the products.

COMMUNICATION

Another issue of concern, the German agency raises, is that the consumer’s right to information on Substances of Very High Concern (SVHCs) in articles, as outlined in Article 33 of REACH, is “hardly feasible” and should be amended by obligatory labelling. The Swedish Chemical Agency (Kemi) calls it a “very weak measure,” in its response to the consultation.

Furthermore, consumer information on substances in mixtures is “missing completely,” says Germany’s environment agency.

Kemi says that while risk communication under EU legislation is good for pesticides and chemical products it is “insufficient” for products for professionals and consumers.

The Dutch Ministry of Health, Welfare and Sport says communication on hazardous substances to consumers is “rather difficult,” and it is doubtful that legislation can solve the problem. The Italian Ministry for Economic Development says consumer associations need to be more involved with communicating safe use of products containing hazardous substances.

CONSUMERS “EATING” MINERAL OIL IN LIP BALM?

Meanwhile, the Danish Consumer Council’s Think Chemicals program found that 14 out of 20 lip balms tested contained potentially carcinogenic mineral oil aromatic hydrocarbons (MOAHs).

Mineral oil saturated hydrocarbons (MOSHs), which were also found, can potentially be “eaten” by users and accumulate in the body’s organs, such as the liver, spleen, and lymph nodes, Think Chemicals says.

In cooperation with German consumer organization Stiftung Warentest, the Danish consumer council found that of the 14 lip balms available on the Danish market, the concentration of MOSH was between 2% and 77%, and MOAH content was between 0.04% and 4.3%.

“It is important to emphasize that the concern for mineral oils applies to lip products, such as lip balms and lipsticks, where it is very likely that you ‘eat’ the oils,” Stine Müller, project manager of Think Chemicals, says.

According to the EU’s Scientific Committee on Consumer Safety (SCCS), daily users will “eat” four lip balms each year, Think Chemicals says.

Koni Grob at the Food Safety Laboratory in Zurich says that frequent use of lip products, with elevated content of MOSH, will be “a very significant source” of exposure. However, he points out that if it makes up less than 5% of the lip balm, the intake will be more limited and the exposure to MOAH will also be low. Müller recommends that if consumers want to avoid mineral oils in lip balms, they can choose products that are based solely on vegetable oils and fats or beeswax.

Ingredients based on mineral oils have different names, Think Chemicals says. The ingredients that contain MOSH and MOAH will be listed on the product ingredients as: petrolatum, paraffinum liquidum, paraffin, cera microcristallina/microcrystalline wax, cerasina, mineral oil, ozokerite, or synthetic wax.

The group says these ingredients are allowed in cosmetics, including lip products, if the manufacturer knows the full refining history, and the ingredients have been purified to remove carcinogenic substances.

In May 2015, a German Federal Institute for Risk Assessment (BfR) report said MOAHs in cosmetic products are unlikely to pose a risk to human health.

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Chile, has recently implemented a new labeling law requiring packaged food products “high in...” calories, saturated fat, total sugars, and/or sodium to have a warning sign. The overall objective of the law is to reduce the incidence of obesity and its related chronic diseases. To learn more about it, I interviewed David Carré, general manager of SoluTec LTDA., and Paula Venegas, technical director at Blumos S.A., in Santiago, Chile.

Q: What does the “Ley Semáforo” / Law 20.606 involve? Is it already enforced?

In response to alarming obesity rates as well as the incidence of non-transmittable chronic diseases in young adults and children, the Chilean government implemented a new law: “Ley Semáforo” (“Stop Light Law,” as it translates from Spanish).

This law aims to provide consumers with the right to knowledge that will help them during the selection of foods for a healthy diet. The overall objective is to reduce caloric consumption, as well as consumption of high levels of total sugars, saturated fats, and sodium. The law establishes specific limits for these nutrients, per 100 grams or 100 mL of packaged food product.

The law was first enacted on June 2015, but it was fully implemented a year later on June 2016. The information is communicated by using an octagonal logo that resembles a stop sign; the logo has a black background with white fonts. Targeted advertising to children 14 years of age or younger is not allowed for foods bearing the stop sign, which indicates that they have a high content of one or more regulated categories. The new law restricts print
or online advertising to young people, and schools are also restricted from selling products with the stop sign. Examples of foods now carrying the stop sign are some sugared beverages, cereals containing ~40% sugars, and other foods generally thought of as “junk foods.”

Q: How does the “Ley Semáforo” pertain to fats in particular?

The law requires labeling of saturated fats per 100 grams or 100 mL of food product. A packaged solid food product would require the “high in...” warning label if it contains 4 grams of saturated fat in 100 grams of product. A packaged liquid food product would require the “high in...” warning label if it contains 3 grams of saturated fat in 100 mL of product.

Trans fats are regulated by a previous law (2014), and should not exceed 2% of the total fats within the packaged food.

Q: As an Associate Member of Mercosur, how does the “Ley Semáforo” affect trade with other Mercosur countries?

As with Chile, the rest of Latin America also has high statistics on obesity and associated chronic diseases; therefore, any move towards a healthier diet is likely to trigger similar moves in other countries. Chile is not a member of Mercosur per se; however, it welcomes many exports from the Mercosur member countries. Those companies importing food products coming from Mercosur must label their products according to the Ley Semáforo. This may be an additional cost that should be considered within the final cost of importation.

Q: Do you think that this law provides opportunities for new food product development, or is it mostly a warning system for the consumer to make conscious choices?

This law provides consumers with knowledge and companies with opportunities to develop new food products that would avoid the warning label. However, in some categories, such as bakery and meats, the challenges could be difficult to overcome. This is because the “high in...” warning label would be removed only if a product does not exceed any of the strict limits on calories and ingredient content. For example, a 100-gram solid food product having 275 kcal, and/or 10 grams total sugars, and/or 400 mg sodium, would have three labels with a “high in calories, sugars, and sodium” warning, respectively. The same product would have to be reformulated to reduce all three categories for the warning label to be removed. More information on the Ley Semáforo can be found at http://web.minsal.cl/wp-content/uploads/2015/08/decreto_etiquetado_alimentos_2015.pdf.
Enzymatic process for fat and oil hydrolysis

Lali, A.M., et al., Institute of Chemical Technology (Deemed University), US9512451, December 6, 2016

An efficient process for enzymatic hydrolysis of fats and oils in a homogenous mixture is provided herein. The present invention in particular provides a process for production of fatty acids, sn-regio mono-acylglycerol (MAG), sn-regio di-acyl-glycerols (DAG), and glycerol from fats, wherein more than 98% fats can be converted into the desired product. The present invention also provides a process for the production of fatty acids and glycerol, virtually free of sn-regio diacyl-glycerols (DAG) and comprising less than 5% sn-regio mono-acylglycerol (MAG) in the end product.

Composition and process for baked food products to impart the sensorial attributes of fried food products

Wang, Y. J., et al., Board of Trustees of the University of Arkansas, US9516885, December 13, 2016

A composition and process for cooked food products is provided to impart the sensorial attributes of fried food products. The composition is in the form of a batter coating, either powdered or wet, which is applied to a food product, and when cooked, has the taste, texture, and appearance of a fried food product. The batter coating includes at least an enzyme-modified starch or flour or other starch-containing material having oil absorbing capabilities. The enzyme-modified starch is plated with a liquid cooking oil in order to bring the liquid cooking oil into the process, and the batter coating retains the incorporated liquid cooking oil during processing to impart a fat fried texture, appearance and taste to the cooked food product.

Bioderived based plasticizers


A bioderived based plasticizer is produced by reacting a bioderived diol (and/or a bioderived alcohol) and a bioderived carboxylic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC), wherein the bioderived carboxylic acid includes a hydrolyzed oil. The bioderived carboxylic acid (e.g., linoleic acid, a-linolenic acid, oleic acid, and mixtures thereof) may be produced by hydrolyzing a triglyceride, such as canola oil, linseed oil, soybean oil, and mixtures thereof. In one embodiment of the present invention, a bioderived based plasticizer is produced by reacting 2,5-bis-(hydroxymethyl)furan and a-linolenic acid in the presence of DCC. In some embodiments of the present invention, the bioderived based plasticizer is blended into one or more polymers.

Cocoa bean processing methods and techniques


Improved methods and/or techniques for processing and/or extracting materials from cocoa beans. In certain embodiments, cocoa bean processing methods (e.g., using unfermented or fermented or roasted or non-roasted beans) which result in cocoa products with improved taste characteristics and/or increased levels of anti-oxidants and/or vitamins.

Pharmaceutical lipid compositions


The present invention relates to a particulate composition containing: a) 5–90% of at least one phosphatidyl choline component b) 5–90% of at least one diacyl glycerol component, at least one tocopherol, or mixtures thereof, and c) 1–40% of at least one non-ionic stabilizing amphiphile, where all parts are by weight relative to the sum of the weights of a+b+c and where the composition contains particles of at least one non-lamellar phase structure or forms particles of at least one non-lamellar phase structure when contacted with an aqueous fluid. The invention additionally relates to pharmaceutical formulations containing such compositions, methods for their formation and methods of treatment comprising their administration.

Catalytic purification of fatty acid alkyl esters used in fuels


The process of this invention removes impurities from transesterification products comprising primarily fatty acid alkyl esters (FAAE) that are being processed for final fuel products, such as biodiesel. The inventive process is catalytic, and the resulting ester is suitable for use as biodiesel. Metal oxide and mixed metal oxide catalysts are particularly suitable. The invention is particularly suitable for treating fatty acid alkyl ester compositions comprising impurities such as glycerin, sterol glycosides, and/or triglyceride, diglyceride and/or monoglyceride. The invention is particularly useful in treating FAAE transesterification products made using homogeneous alkali catalysts. The treated ester exhibits improved performance under cold weather conditions, which can be measured by methods such as ASTM 7501 Cold Soak Filtration Test (CSFT).

Metabolic imprinting effects of specifically designed lipid component


The invention relates to the use of specifically designed lipid component with optimal fatty acid profile, an enhanced portion of the palmitic acid residues in the sn-2 position, and present as lipid
globules with a phospholipid coating for an early in life diet for improving the development of a healthy body composition, in particular prevention of obesity, later in life.

Methods of direct addition of (meth)acrylic acid to bio-based oils


(Meth)acrylates are prepared in a single-step method from a mixture of (meth)acrylic acid and at least one biobased oil and/or its derivative(s), including at least one unsaturation. The (meth)acrylates are made by directly adding the (meth)acrylic acid to the biobased oil by reacting in the presence of an acid catalyst, including an inorganic or organic acid having at least one oxygen atom present thereon and which possesses at least one acid functionality having an ionization constant in water which is not greater than 3.

Hydraulic fluids and fire-resistant fluids comprising glycerin-containing by-products

Tran, B.O., et al., Nalco Co., US9534189, January 3, 2017

Hydraulic fluid and fire-resistant fluid compositions and methods of using the compositions are provided. In an embodiment, the present invention provides a method of utilizing fire-resistant fluid in hydraulic systems. For example, the method can comprise utilizing fire-resistant fluid in hydraulic systems of casters found in steel mills, in an environment where fire safety is of concern. The fire-resistant composition can comprise one or more glycerin by-products derived from a biodiesel manufacturing process. The fire-resistant composition can also comprise one or more glycerin by-products of transesterification reactions involving triglycerides. The fire-resistant fluid can be added to hydraulic systems as a solution.

Low-saturated-fat sunflower and associated methods

Gerdes, J.T., et al., Dow AgroSciences LLC, US9538715, January 10, 2017

Provided are sunflowers, parts thereof, cultures of, and seeds that are capable of producing sunflower oil that is low in saturated fat and, optionally, high in linoleic acid as well as associated methods.

Emulsion

Haug, I., et al., Ayanda Group AS, January 10, 2017

The invention relates to an orally administrable chewable composition in unit dosage form comprising an oil-in-water emulsion in which the aqueous phase is gelled and in which the oil phase comprises a physiologically tolerable unsaturated fatty acid ester.

Energy-curable news ink containing soy oil


An energy curable ink composition comprising an acrylate-functional derivative of soybean oil and one or more further acrylate-functional materials is suitable for printing on a cold-set lithographic press and is cured by exposure to an actinic radiation source located on the press.

Oxidized mixture of bio-based material and asphalt, a bio-based asphaltic material, and methods of making the same


A method of forming an asphalt mixture can include mixing a bio-source material and a bitumen source to form a bitumen mixture. The bitumen mixture can be mixed with a catalyst to form the asphalt mixture. Particles can be added to the asphalt mixture to form a roofing-grade asphalt mixture. In an embodiment, the bitumen source material can have a softening point of at least approximately 93°C and a penetration distance no greater than approximately 25 dmm. In another embodiment, the roofing-grade asphalt mixture can have a softening point of at least approximately 104°C, a penetration distance no greater than approximately 12 dmm, a viscosity of at least approximately 3000 cps at a temperature of 204°C, or any combination thereof. The asphalt mixture can be applied to a base material to form a roofing product. The asphalt mixture can be applied as a pavement product.

Lipase powder compositions

Negishi, S., et al., The Nisshin Oillio Group, Ltd. and Novozymes A/S, US9550961, January 24, 2017

The present invention provides a lipase powder composition which comprises a filter aid(s) and a product obtained by pulverizing a Thermomyces sp.-derived lipase immobilized to a silica carrier(s) into the average particle diameter of 1 µm or more and less than 300 µm. This lipase powder composition improves the lipase activity and operability and, therefore, can be suitably used in the methods for exchanging esters of fats and oils and for esterification.

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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.
JSD celebrates 20th volume with selected articles: 3 more summaries

Five articles have been selected in recognition of the Journal of Surfactants and Detergents' 20th anniversary year. The articles were chosen because they were either highly cited, most-downloaded, of general significance, or cover investigations important to surfactants and detergents researchers. Two of the articles were summarized by JSD’s Editor-in-Chief, George A. Smith in the February issue. Here are his summaries of the other three. Full versions of all five articles are available at www.springer.com/jsd.

ENVIRONMENTALLY FRIENDLY VEGETABLE OIL MICROEMULSIONS USING EXTENDED SURFACTANTS AND LINKERS


Formulation of triglyceride microemulsions under ambient temperature conditions is very difficult without the use of co-solvents to control gel phase formation. Microemulsions of different common triglycerides have been prepared using a combination of extended chain surfactants and linkers. Different triglycerides include olive, peanut, soybean, canola, and sunflower oils. Extended chain surfactants studied were propoxy ether sulfates with oleyl alcohol as a lipophilic linker and substituted naphthalene sulfonates and polyglucosides as hydrophilic linkers. Depending on the salinity conditions, Winsor type I, II, III, and IV microemulsions have been prepared, and the phase behavior has been shown in “fish” diagrams. Winsor type III and IV microemulsions are useful in several applications including cosmetics, vegetable oil extraction, and soil remediation.

DETERMINATION OF CRITICAL MICELLE CONCENTRATION (CMC) OF NONIONIC SURFACTANTS BY DONOR-ACCEPTOR INTERACTION WITH IODINE AND CORRELATION OF CMC WITH HYDROPHILE-LIPOPHILE BALANCE AND OTHER PARAMETERS OF THE SURFACTANTS


A new technique for measuring critical micelle concentration (CMC) based on complexation with iodine is reported. Nonionic surfactants form donor-acceptor complexes with iodine in aqueous solution. The shift in $\lambda_{\text{max}}$ upon complexation can be used to measure the CMC of different nonionic surfactants including polysorbates, octylphenol ethoxylates, and alcohol ethoxylates. The measured CMC values agree closely with results obtained from surface tension and the more traditional iodine solubilization method. The CMC values can be correlated with the hydrophile-lipophile balance (HLB). Both the CMC and HLB were found to be temperature dependent.

ON THE MEASUREMENT OF CRITICAL MICELLE CONCENTRATIONS OF PURE AND TECHNICAL-GRADE NONIONIC SURFACTANTS


The critical micelle concentration (CMC) of commercial nonionic surfactants was determined by surface tension and dye solubilization methods. Commercial nonionic surfactants have a broad molecular weight distribution and usually contain various surface active impurities. Surface tension measurements are very sensitive to impurities and can show much lower CMC values than dye solubilization. Surface active impurities can saturate the air-water interface well below the CMC which influences the break in the surface tension versus concentration plot. Dye solubilization measures the spectral shift when dye molecules are solubilized in micelles and is not affected by impurities. Pure isomeric nonionic surfactants and commercial nonionics purified by foam fractionation to remove the impurities show the same CMC by both techniques. Precaution should be taken when measuring the CMC of commercial nonionic surfactants containing surface active impurities by surface tension measurements.
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Microdialysis as a new technique for extracting phenolic compounds from extra virgin olive oil


The amount and composition of phenolic components play a major role in determining the quality of olive oil. The traditional liquid–liquid extraction (LLE) method requires a time-consuming sample preparation to obtain the “phenolic profile” of extra virgin olive oil (EVOO). This study aimed to develop a microdialysis extraction (MDE) as an alternative to the LLE method to evaluate the phenolic components of EVOO. To this purpose, a microdialysis device and dialysis procedure were developed. “Dynamic-oil” microdialysis was performed using an extracting solution (80:20 methanol/water) flow rate of 2 μL min⁻¹ and a constant EVOO stream of 4 μL min⁻¹. The results indicated a strong positive correlation between MDE and the LLE method, providing a very similar phenolic profile obtained with traditional LLE. In conclusion, the MDE approach, easier and quicker in comparison to LLE, provided a reliable procedure to determine the phenolic components used as a marker of the quality and traceability of EVOO.

Rapid detection and separation of olive oil and Camellia oil based on ion mobility spectrometry fingerprints and chemometric models


A simple and rapid classification model for olive and Camellia oil was proposed based on ion mobility spectrometry (IMS) fingerprints and chemometric model (peak detection and random forest algorithm). Results indicated that IMS fingerprint spectra by second-derivative algorithm could completely separate 64 olive oil and 79 Camellia oil samples used in this study by simply calculating the peak area. Random forest algorithm was employed to establish discriminant model for olive oil adulterated by Camellia oil. Simulated adulteration detection showed that the accuracy rate of discriminant model is 96.4% as two of 55 samples were identified as blending olive oil. All these results suggested that IMS could be an effective method to detect the adulterated olive oils by Camellia oil.

Influence of deodorization temperature on formation of tocopherol esters and fatty acids polymers in vegetable oil


The paper describes laboratory deodorization of rapeseed, model sunflower, and model rapeseed oils, and its effect on the tocopherol content and the kinetics of tocopherol degradation. We present a novel approach to analyzing alpha-tocopherol degradation products as well as kinetic study esters of alpha-tocopherol with fatty acids and alpha-tocopheryl quinones. Alpha-tocopherol esters were found to be the principal products of tocopherols degradation in a high-temperature experiment. A laboratory deodorization, lasting 2 h caused formation of 70–128 μg/kg of alpha-tocopherol esters in model oils and 25 μg/kg of alpha-tocopherol esters in rapeseed oil without modification. The commercially available sunflower and rapeseed oils contained 3–12 mg/kg of alpha-tocopherol esters. The alpha-tocopherol esters are formed at higher rate than alpha-tocopheryl quinone, which was unsta-
ble at high deodorization temperature. Tocopherols took no part in non-radical thermal reactions, taking place in second high-temperature experiments with oxygen-free atmosphere. We proved by DART TOF-MS the formation of polymeric fatty acids via Diels–Alder reaction taking place in heated oils.

Development and validation of a novel microwave-assisted extraction method for fish lipids


A novel microwave-assisted extraction (MAE) method for fish lipids was optimized by means of a central composite design and validated using a fish tissue standard reference material (SRM, 1946, NIST). Scanning electron microscopy showed that MAE caused total disruption of the fish tissue, thus lipid migration to the extraction solvent was more efficient and faster in comparison to the Folch method. The lipid content of the SRM obtained by MAE (10.1 ± 0.2 g/100 g) was similar to that declared on the certificate (10.2 ± 0.5 g/100 g). The use of microwave energy did not alter the fatty acid composition of the fish nor formed lipid oxidation derived compounds at higher levels than in the Folch method. The MAE method was applied to fishes with different lipid contents, tilapia, pacu, and hake. MAE is a fast and robust technique with low solvent consumption when compared to the Folch method.

Synthesis of high-quality biodiesel using feedstock and catalyst derived from fish wastes


A low-cost and high-purity calcium oxide (CaO) prepared from waste crab shells was used as an efficient solid base catalyst in the synthesis of biodiesel. Raw fish oil was extracted from fish waste using a mechanical expeller followed by solvent extraction. Physical as well as chemical properties of raw fish oil were studied, and its free fatty acid composition was analyzed with GC-MS. Stable and high-purity CaO was obtained when the material was calcined at 800°C for 4 h. Prepared catalyst was characterized by XRD, FT-IR, and TGA/DTA. The surface structure of the catalyst was analyzed with SEM, and elemental composition was determined by EDX spectra. Esterification followed by transesterification reactions were conducted for the synthesis of biodiesel. The effect of co-solvent on biodiesel yield was studied in each experiment using different solvents such as toluene, diethyl ether, hexane, tetrahydrofuran, and acetone. High-quality and pure biodiesel was synthesized and characterized by 1H NMR and FT-IR. Biodiesel yield was affected by parameters such as reaction temperature, reaction time, molar ratio (methanol:oil), and catalyst loading. Properties of synthesized biodiesel such as density, kinematic viscosity, and cloud point were determined according to ASTM standards. Reusability of prepared CaO catalyst was checked, and the catalyst was found to be stable up to five runs without significant loss of catalytic activity.

Effect of furan fatty acids and 3-methyl-2,4-nonanedione on light-induced off-odor in soybean oil


Soybean oil is one of the most widely consumed vegetable oils. However, under photooxidative conditions, this oil develops a beany and green off-odor through a mechanism that has not yet been elucidated. Upon photooxidation, 3-methyl-2,4-nonanedione (3-MND) produces a strong aroma. In this study, the effect of furan fatty acids and 3-MND on odor reversion in soybean oil was investigated. Our findings suggest that the observed light-induced off-odor was likely attributable to the furan fatty acids present in the oil through the generation of 3-MND. While 3-MND may not be directly responsible for the development of light-induced off-odor, this compound appears to be involved because off-odor was detected in canola oil samples containing added 3-MND. In addition, in the present work, 3-hydroxy-3-methyl-2,4-nonanone, which is derived from 3-MND, was identified for the first time in light-exposed soybean oil and shown to be one of the compounds responsible for odor reversion.
Comparison of the impact of SFAs from cheese and butter on cardiometabolic risk factors: a randomized controlled trial


Controversies persist concerning the association between intake of dietary saturated fatty acids (SFAs) and cardiovascular disease risk. We compared the impact of consuming equal amounts of SFAs from cheese and butter on cardiometabolic risk factors. In a multicenter, crossover, randomized controlled trial, 92 men and women with abdominal obesity and relatively low HDL-cholesterol concentrations were assigned to sequences of 5 predetermined isoenergetic diets of 4 wk each separated by 4-wk washouts: 2 diets rich in SFAs (12.4–12.6% of calories) from either cheese or butter; a monounsaturated fatty acid (MUFA)-rich diet (SFAs: 5.8%, MUFAs: 19.6%); a polyunsaturated fatty acid (PUFA)-rich diet (SFAs: 5.8%, PUFAs: 11.5%); and a low-fat, high-carbohydrate diet (fat: 25%, SFAs: 5.8%). Serum HDL-cholesterol concentrations were similar after the cheese and butter diets but were significantly higher than after the carbohydrate diet (+3.8% and +4.7%, respectively; P < 0.05 for both). LDL-cholesterol concentrations after the cheese diet were lower than after the butter diet (-3.3%, P < 0.05) but were higher than after the carbohydrate diet (+2.6%), MUFA (+5.3%), and PUFA (+12.3%) diets (P < 0.05 for all). LDL-cholesterol concentrations after the butter diet also increased significantly (from +6.1% to +16.2%, P < 0.05) compared with the carbohydrate, MUFA, and PUFA diets. The LDL-cholesterol response to treatment was significantly modified by baseline values (P-interaction = 0.02), with the increase in LDL cholesterol being significantly greater with butter than with cheese only among individuals with high baseline LDL-cholesterol concentrations. There was no significant difference between all diets on inflammation markers, blood pressure, and insulin-glucose homeostasis. The results of our study suggest that the consumption of SFAs from cheese and butter has similar effects on HDL cholesterol but differentially modifies LDL-cholesterol concentrations compared with the effects of carbohydrates, MUFAs, and PUFAs, particularly in individuals with high LDL cholesterol. In contrast, SFAs from either cheese or butter have no significant effects on several other nonlipid cardiometabolic risk factors. This trial was registered at clinicaltrials.gov as NCT02106208.

Environmental impacts in the life cycle of olive oil: a literature review


The production of olive oil is considered to be one of the largest agricultural business sectors in the Mediterranean area. Apart from its significant impact on the economies of countries in Southern Europe, Northern Africa, and the Middle East, olive oil production also involves considerable social and environmental considerations. However, despite such importance, the environmental effects of olive oil production have not been studied as much as other agricultural productions and farming systems, which are more characteristic of central and northern Europe. We present a thorough and systematic literature review of scientific publications with respect to the use of environmental tools in the life cycle of olive oil. The analysis takes into consideration the farming of olive trees, the manufacture of olive oil, packaging, transportation, and reverse logistics. To that end, journal publications up to 2015 in this specific field are recorded and, at the same time, the most important environmental impacts are revealed and a gap analysis conducted. The analysis conducted reveals that farming of olive trees (with pesticide use and waste/by-product production being the “hottest” topics) and the manufacturing of olive oil (concentrating mostly on waste/by-product production and management) are the phases with the highest environmental focus from the scientific community. Moreover, gaps in the literature are detected mostly with respect to fuel consumption and the use and promotion of renewable energy sources in olive oil production.

Converting defatted silkworm pupae by Yarrowia lipolytica for enhanced lipid production


The valorization of converting defatted silkworm pupae (DSWP) into microbial lipids by the oleaginous yeast Yarrowia lipolytica was investigated. To obtain the best hydrolysis of DSWP and obtain the most final production after cultivation, the method of ultrasound-assisted enzymatic pretreatment in the presence of 1-butyl-3-me-
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Hydrolysis was applied to pretreat the DSWP. Under the optimal conditions by response surface methodology, the highest weight loss achieved 84.97%, the hydrolysis degree (32.3%) and lipid yield (3.71 g/L) have increased by 6.0- and 1.3-fold, respectively. When adding amount of defatted silkworm pupae hydrolysate (DSWPH) was 4 g/L, the yeast could produce lipid efficiently. Besides, the cost is much lower than the peptone. The replacement of peptone with DSWP for lipid production by Y. lipolytica W29 efficiently cut down the cost of microbial lipid production. The lipid concentration of yeast grew on medium with 4 g/L DSWPH adding amount has increased by threefold compared with control group, while the citric acid concentration was decrease to 0.27-fold. Given the large amount of fatty acids accumulation, instead of citric acid accumulation, the DSWPH could replace the industrial nitrogen source in the future.

**Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste**


This study aimed at evaluating the oils extracted from seeds originating from agroindustrial wastes to identify the presence of bioactive compounds. The oils extracted from seeds of apple, citrus, grape, guava, kumquat, mango, melon, orange, papaya, passion fruit, pumpkin, sour sop, strawberry, and tomato were studied. Therefore, determination of fatty acid profile, triglycerides, composition of tocopherols, phytosterols and phenolic compounds, and total carotenoids was performed in the oils. Strawberry seed oil stood out for its high content of linolenic acid (31.5%), which is classified as essential fatty acid, and also for its high content of phenolic compounds, compared to the other samples. The concentrations of tocopherols ranged from none detected in kumquat seed oil to 54.53 mg/kg in apple seed oil. The main phytosterol found among the samples was β-sitosterol.

**Industrial Applications**

**In-situ transesterification process for biodiesel production using spent coffee grounds from the instant coffee industry**


Industrial spent coffee grounds (IND-SCG) are a potential non-edible biodiesel feedstock due to their abundant global supply and high oil content. In this study, an *in-situ* transesterification (*in-situ* TE) was developed and scaled up for IND-SCG biodiesel production. Several hurdles must be overcome, including the high acid value, and wide range in particle size of IND-SCG. Washing IND-SCG with methanol reduced its high acid value with negligible loss of oil. Size reduction (0.25–1.68 mm) and an increase of the reaction temperatures (30–60 °C) were found to improve the biodiesel yield significantly. The whole deacidified IND-SCG was processed at 50 °C, and a maximum biodiesel yield of 77% was achieved within 3 h. The process was successfully scaled up for processing 4 kg IND-SCG per batch with a yield comparable to the 30-g scale. The IND-SCG biodiesel met the ASTM biodiesel standard in terms of total glycerin, water content, kinematic viscosity, and oxidative stability index (OSI), but its acid value exceeded the standard. A simple process modification using acidic water to neutralize alkaline catalyst during refining step, instead of strong acid, enabled the IND-SCG biodiesel to meet the standard for acid value. The oxidative stability index of the *in-situ* IND-SCG biodiesel was superior to that of the conventional process, probably due to the co-extraction of natural antioxidants.

**A method of producing edible oils with high quality by water**


Environmental, health, and cost concerns have triggered great interest in developing an aqueous method to process oil seeds. Here we describe a method of processing oil seeds using a small amount of water, which has a higher recovery rate of first- or second-class oils as compared with high-pressure pressing and solvent extraction. The method utilizes all the kernels of oil seeds and does not produce waste water or other wastes. This breakthrough indicates the strong potential of replacing solvent extraction or high-pressure pressing for oilseed processing by the aqueous method.

**The potential of organic solvent nanofiltration processes for oleochemical industry**


Pressure-driven membrane separation processes bare the promise to significantly reduce energy requirements and operational cost compared to classical thermal separation processes. The ability of organic solvent nanofiltration (OSN) to operate at mild temperatures makes it especially interesting for oleochemical processes, such as the refinement of non-edible oils or waste oils. In this study, the potential of OSN for solvent recovery and deacidification is investigated by means of a model-based process analysis. On the basis of optimized membrane cascade configurations, the OSN process is compared to the conventional reference process in terms of energy requirements and costs to judge on the competitiveness. It is shown that the energy demand for the recovery of extraction solvents can be reduced by more than 70% using an OSN-assisted evaporation process. While the operating costs are significantly reduced, the investment costs are increased in comparison to a classical evaporation process. The process analysis also shows that OSN is a promising alternative for deacidification of various low-quality oils using multi-stage membrane cascades. Optimal process design makes it possible to upgrade the triglycerides to the necessary purity (e.g., for processing them in biodiesel production), while the free fatty acids are recovered as valuable by-products that enhance the profitability. Hence, OSN constitutes a valuable processing technology that can be integrated into upstream separation and recovery processes of the oleochemical industry.
Karen Letourneau has worked at POS Bio-Sciences for 26 years and POS Analytical Services has had an AOCS Approved Chemist on staff since 2011. “Being part of the AOCS Lab Proficiency Program really improves the quality of our testing.”

Letourneau explains that although POS Bio-Sciences had been performing marine oil lab testing for years, the laboratory was striving for greater accuracy and consistency. “Comparing our data to other labs’ data helped us improve, and we used the check samples obtained through AOCS to train our technicians,” she says. “Our efforts paid off and we’ve placed first in the AOCS Marine Oil series.”
PUFA synthases produce a limited set of fatty acids as their primary products, including DHA, EPA, and docosapentaenoic acid (DPA n-6, 22:5 n-6). This list was recently expanded to include arachidonic acid (ARA, 20:4 n-6) being produced in a marine bacterium, *Aureispira marina* as well as linoleic acid in a myxobacterium (LA, 18:2 n-6). Most of these fatty acids are typically associated with primary metabolism in animals rather than the secondary metabolites normally produced by PKS systems. Depending on the specific PUFA synthase, the main product can be a single PUFA such as DHA, EPA, or ARA, or a mix of PUFAs such as DHA and DPA n-6 or DHA and EPA. All of these fatty acids are 18 to 22 carbons in length and all have between 2 and 6, methylene interrupted, carbon-carbon double bonds. These double bonds are always in the *cis* configuration, and the first double bond occurs at either the n-3 or n-6 position. The important roles of some of these PUFAs, especially DHA, EPA, and ARA, in human physiology have been well documented. Why these particular PUFAs should be made and accumulate in microorganisms remains an open question. Evidence that PUFAs play essential roles in some of these microorganisms was demonstrated by the dependence on supplemental PUFAs for growth of strains of *Schizochytrium* sp. ATCC 20888 in which the PUFA synthase had been inactivated.

**BACTERIAL PUFA SYNTHASES**

Genes encoding PUFA synthases were first discovered in marine bacteria. Contrary to prevailing views of the time, DeLong *et al.* established that certain marine bacteria contained significant levels of PUFAs, such as EPA and DHA. In a remarkable achievement, Yazawa was able to use a cosmid derived from one of those strains (*Shewanella* strain SCRC-2378, presently identified as *Shewanella pneumatophori*) to produce EPA in *E. coli*. EPA was the only new fatty acid produced in the *E. coli* transformants and subsequent work revealed that five open reading frames (ORFs) present in the cosmid were necessary and sufficient for EPA accumulation in the heterologous host. It was then proposed in 2001 that the proteins encoded by four of these ORFs represented subunits of a novel Type I enzyme capable of *de novo* PUFA synthesis. This hypothesis was based on a number of factors, including: examination of the domain functions, *in vitro* activity assays, and the observation that PUFA synthesis could occur in the absence of *O*₂. The fifth ORF, identified as essential for EPA syn-

Polyunsaturated fatty acid (PUFA) synthases are Type I iterative enzymes that carry out *de novo* synthesis of specific long-chain PUFAs using malonyl-CoA as the carbon source. The products of these synthases include three PUFAs that are of primary interest to human health and nutrition: docosahexaenoic acid (DHA, 22:6 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and arachidonic acid (ARA, 20:4 n-6). PUFA synthases are primarily found in marine bacteria and the thaustochytrids, a group of marine heterokont microalgae. Recently, the range of microorganisms containing PUFA synthases has been expanded to include examples from fresh water bacteria, terrestrial myxobacteria, and possibly another type of eukaryotic microalga (*Emiliania huxleyi*).

Unlike the “standard pathway” in which a series of elongase and desaturase reactions generate PUFAs from pre-existing, short-chain fatty acids, PUFA synthases do not have a requirement for molecular oxygen for insertion of the carbon-carbon double bonds and the synthesis mechanism is sometimes referred to as the “anaerobic pathway.”

PUFA synthases are often referred to as a polyketide synthase (PKS) system since several of their enzymatic domains have significant homology to those typically found in Type I PKSs. But ‘PKS-like’ would be more appropriate since PUFA synthases possess unique features that distinguish them from previously described systems. They are hybrid systems incorporating elements of Type I PKS and of Type II PKS and FAS systems. For example, several of the enzymatic domains embedded in the multi-domain subunits of PUFA synthases are homologous to those typically found only as discreet components of Type II PKS systems or of Type II fatty acid synthases (FASs). These include two tandem domains with homology to a dehydratase/isomerase (DH/I) found in some Type II FAS systems, the FabA-like enzymes, and a tandem pair of domains with homology to the β-ketocycl synthase (KS)—chain-length factor (CLF) heterodimeric enzyme found in Type II PKS systems. CLFs are proteins with homology to KSSs but lacking the active site residues. Additionally, all PUFA synthases identified to date contain from 3 to 10 tandem acyl-carrier protein (ACP) domains.

**Text excerpted from http://lipidlibrary.aocs.org/Biochemistry/content.cfm?ItemNumber=41528; full version includes figures and references.**

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

Ross Zirkle and Jim Metz
thesis, was found to encode an accessory enzyme required for
activation of the ACP groups of the PUFA synthase enzyme by
attachment of a co-factor by a phosphopantetheinyl trans-
ferase (PPTase). Although *E. coli* contains several endogenous
PPTases, they were apparently unable to recognize and activ-
ate the ACP domains of the *Shewanella* PUFA synthase.

Homologous gene sets encoding PUFA synthase subunits
have been identified in numerous other marine bacteria. In
several cases, these homologs have been confirmed as being
PUFA synthases by heterologous expression and detection of
novel PUFAs in the new host, including the production of ARA
and LA. While variation in the domain content and organiza-
tion has been observed, including additional putative KS and
FabA-like DH domains, the key distinguishing features are still
apparent in the verified PUFA synthases. These examples indi-
cate that most of PUFAs produced in bacteria are likely the
products of PUFA synthase systems.

**EUKARYOTIC PUFA SYNTHASES**

Concurrent with the proposal of a bacterial PUFA syn-
thase system, a homologous system was characterized in
a eukaryotic microalga that had been developed as a com-
mmercial source of oil enriched in DHA: *Schizochytrium* sp.
ATCC 20888. *Schizochytrium* sp. is a member of the thraus-
tochytrids, a group of large-celled heterokont protists.
Subsequently, PUFA synthase genes have been found in sev-
eral other Thraustochytrids. Presumably the thraustochytrids
acquired PUFA synthase genes from marine bacteria via lateral
gen transfer. The domains of the thraustochytrid PUFA syn-
thases are organized somewhat differently than most of the
bacterial versions. Typically there are three subunits, instead
of four, and there is a duplication of the enoyl-reductase (ER)
domain. While the bacterial PUFA synthase subunit genes are
organized as part of an operon, separate genes encode the
subunits of the eukaryotic thraustochytrid synthases.

It is noteworthy that, in the cases where it has been exam-
ined, *PFA1* and 2 are adjacent in a head-to-head arrange-
ment. Several thraustochytrids that possess PUFA synthases
have also been shown to contain a partial, or complete, set
of enzymes of the standard PUFA synthesis pathway (*i.e.*, the
elongase and desaturase enzymes). In some cases the stan-
dard pathway is functional, including production of DHA. In
the case of *Schizochytrium* sp. ATCC 20888, a partial set of
standard pathway enzymes was identified but a critical Δ12
desaturase activity is missing and this system is therefore not
capable of synthesizing PUFAs from the shorter saturated fatty
acid products of the endogenous FAS. Furthermore, inacti-
vation of the PUFA synthase in this organism results in aux-
otrophy and supplementation of the medium with PUFAs is
required for growth.

Genes that encode proteins with homology to PUFA
synthases continue to be identified. A recent example was
the discovery of a homolog in the eukaryotic coccolitho-
phore, *Emiliania huxleyi*. In this case, all of the domains of the
putative PUFA synthase were encoded on a single large pro-
tein. *E. huxleyi* is a major component of the of the global phy-
toplankton community and is known to produce significant
levels of PUFA. As in the case of other eukaryotes that pos-
sess a PUFA synthase system, *E. huxleyi* also contains a suite of
elongase and desaturase enzymes of the standard PUFA syn-
thesis pathway. It has yet to be determined what the relative
contributions of the two pathways for synthesis of the PUFAs
in this organism may be.

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  - Penny Kris-Etherton is Distinguished Professor of Nutrition in the Department of Nutritional Sciences at The Pennsylvania State University.
  - Peter Meikle is a NHMRC Senior Research Fellow. He is leader of the Metabolism Program and Head of the Metabolomics Laboratory at the Baker Heart and Diabetes Institute.
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