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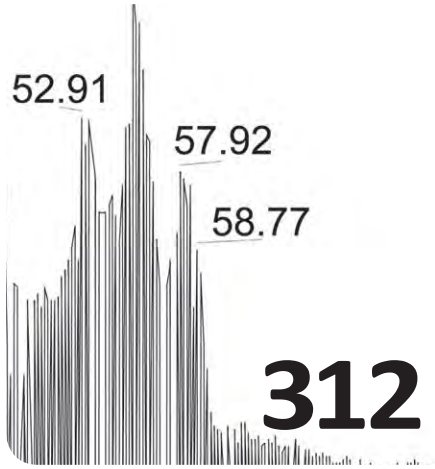

DEPARTMENTS

Letter to the Editor

Dear Editor,

You start the Biofuels news section of the March 2013 issue of *Inform,* page 149, with a report on "Energy from Vietnamese fish wastes."

These wastes can amount to some 80 metric tons per day and this could eventually generate some 13 MT of biodiesel per annum, a yield of just over 0.04%. This strikes me as too low to bother about, but there is another aspect I want to raise: Converting triglyceride oil to biodiesel (fatty acid methyl esters), followed by fueling a generator with this biodiesel to produce electricity, is a roundabout and inefficient way of using the energy content of the raw material. It would be more efficient to burn the oil as such in a power station. This would avoid having to convert the oil into biodiesel and would generate more electricity from a given amount of oil.

In fact, this inefficiency applies not only to this relatively small amount of biodiesel made from fish waste in Vietnam but to biodiesel in general. I heat my house by burning domestic fuel oil in my central heating boiler. This oil is chemically almost identical to diesel fuel. Heating my house with triglyceride oil instead would also obviate the conversion into biodiesel and thus save on conversion costs. As long as domestic fuel oil usage exceeds biodiesel production, this would be a better way of using renewable fuel sources. It might require a small adjustment in my boiler and, depending on the triglyceride oil source, I might have to install a small heater in my fuel tank, but that would be far cheaper than the cost of developing and distributing biodiesel.

Another way of using triglyceride oil as a renewable feedstock is to feed it to an ethylene cracker. Global ethylene capacity is about 140 million metric tons, which is close to the global production of edible oils. Since the ethylene yield is less than 50%—the other products being propylene, butadiene, petrol, benzene, etc.—there will be no problem in accommodating what triglyceride oil is available. It does not even have to be purified as in biodiesel production.

Is the promotion of biodiesel another example of the lack of chemical awareness and insight with the powers that be? It could be. After all, for a politician who has promoted biodiesel, the notices at gas stations about this biodiesel remind the public permanently of what he (or she) has done and achieved: wasted money.

Sincerely,
Albert Dijkstra
Carbougnères, Saint Eutrope-de-Born, France

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EATING COTTON

the saga of ultra-low-gossypol cottonseed

Catherine Watkins

Twenty million farmers in 80 countries grow cotton each year for its fiber. They produce enough cottonseed—a good source of plant protein at about 22.5% by weight—to meet the daily protein requirements of half a billion people per year.

There's just one catch: Only ruminant animals can tolerate cottonseed meal made from most commercial varieties because it contains gossypol, a phenolic toxin found scattered throughout the seed, leaves, stems, and roots of the cotton plant in structures known as pigment glands. Gossypol glands serve a purpose, because the toxin protects the plant from insects and microbes. The challenge that has stumped researchers for decades is how to remove the gossypol—either through processing, traditional plant breeding, or bioengineering—without compromising the vigor of the plant or the quality and quantity of the fiber and by-products such as meal.

"We've been chasing this rabbit for 50 to 60 years," says Tom Wedegaertner of Cotton Incorporated (Cary, North Carolina, USA). Cotton Incorporated is the entity created in 1970 as a result of the Cotton Research and Promotion Act of 1966. The Act established a funding mechanism, based on producer assessments, with the aim of recapturing cotton's market share after synthetic fibers became popular. As Cotton Incorporated's director of cottonseed research and marketing since 1993, Wedegaertner has had a front row seat from which to observe the many and varied attempts to commercialize traditionally bred glandless (gossypol-free) cotton and to create a bioengineered plant with seeds that are free of gossypol.

Could the rabbit finally be in sight? Wedegaertner thinks so, suggesting that the US industry is 6–10 years away from planting a million acres (more than 400,000 hectares) of ultra-low-gossypol biotech cotton. The road from initial characterization of mutant gossypol-free cotton to a new bioengineered variety is a fascinating story of persistence, with an end yet to be written. But if Wedegaertner is right—and if a commercial partner takes up the cause—the developing world will gain another protein source even as growers and processors gain added value.

A BRIEF HISTORY

Researchers identified a gossypol-free mutant cotton strain (Hopi Moencopi, named after a Native American village) in the 1950s. The trait was transferred into commercial varieties through traditional breeding, as the scientific and agricultural community excitedly imagined the many benefits of toxin-free seed, oil, and meal.

Nutritional studies done during the 1960s through 1980s confirmed that glandless cottonseed meal was suitable for monogastric animals and human beings. Scientists at Texas A&M University (TAMU; College Station, USA) and elsewhere showed that roasted, salted glandless cottonseed kernels made an appetizing snack. In 1974, the US Food and Drug Administration set the maximum allowable level of free gossypol in edible food products at 450 parts per million (ppm).

In the midst of the excitement, one major problem remained: Glandless cotton varieties—minus the protective polyphenols of glanded cotton—provided a feast for insects and were, as a result, a commercial failure. (Even rats, which typically do not dine on cottonseed, found the glandless cottonseeds to be particularly tasty.) Processing, quality control, and a lack of viable markets for the oil and protein also worked against successful commercialization.

BIOTECHNOLOGY TO THE RESCUE

And so began the second phase of work: Researchers at various institutions in several countries (most notably the United States, Australia, and China) looked to the new tools of bioengineering to transform the cotton plant. The first studies on bioengineered low-gossypol cotton appeared in the mid-1990s; the research that is currently being funded by Cotton Incorporated—and has Wedegaertner thinking the elusive rabbit may be in sight—has been conducted by Keerti S. Rathore and his team at TAMU.

Rathore, a professor in the department of soil and crop sciences, came to TAMU in 1995 from Purdue University and had never seen a cotton plant growing in the field before taking up residence in Texas. Senior-level researchers at TAMU presented to him the elimination of gossypol as a problem that would be worth solving. One immediate challenge for Rathore, however, was that cotton is a difficult plant to transform, requires a high degree of tissue culture skills, and involves "two distinct and equally important steps," in his words.

CONTINUED ON PAGE 281

- **Cottonseed could be a good source of vegetable protein for developing countries, but only ruminant animals can tolerate gossypol, the phenolic toxin found in pigment glands located in the seeds, leaves, branches, and roots.**
- **Traditionally bred glandless cotton failed in the marketplace several decades ago because without the protection of gossypol, insects devoured the plants. Processing, quality control, and marketing issues also worked against successful commercialization.**
- **Now, researchers at Texas A&M University have used RNAi technology to bioengineer cotton lines that exhibit ultra-low gossypol content in the seeds and normal levels elsewhere. Has the gossypol problem in cottonseed finally been solved?**

New product research in glandless cotton stands to benefit transgenic cotton

New crop market dynamics are a familiar story: Food companies will not develop a new product using an ingredient that does not have a stable supply and seed companies are loath to subsidize development without a ready market.

A multidisciplinary team of researchers at New Mexico State University (NMSU) in Las Cruces, USA, has been working for several years—funded by Cotton Incorporated—to develop a cycle of use for traditionally bred glandless cotton and to conduct field evaluations of insect susceptibility. Their product development work also stands to benefit the ultra-low-gossypol transgenic cotton developed at Texas A&M University (see main story).

NMSU scientists set up a pilot plant where, after harvesting, delinting, and dehulling, they press the oil and make different cottonseed meals and flours for testing new products. The campus foodservice company uses the oil for frying. It is then recycled into biodiesel, which runs a utility vehicle on the farm. Leftover glycerine goes into bar soap.

Of all the applications NMSU has tested, one of the most promising potential markets for glandless cottonseed meal (G-CSM) is in shrimp feed. "We reduced the cost of shrimp meal by almost half," says Tracey Carrillo, assistant director of Campus Farm Operations and superintendent of the Leyendecker Plant Science Research Center at NMSU.

"We could take it even further down by adding algae in place of commercial fishmeal."

The campus dining hall at New Mexico State University (NMSU) supplies used cottonseed cooking oil for conversion into biodiesel that is then used to run the catering vehicle on campus and a new utility vehicle at the farm. The biodiesel processor and both vehicles were donated to NMSU by Cotton Incorporated, which also helps fund cotton research projects at the university. (NMSU photo by Jay A. Rodman)

NMSU food technologists have developed snack foods using G-CSM, Carrillo notes, including an extruded product made of corn flour and G-CSM known as Chiletones. Cookies are next on the product development list, he said.

Research also continues at two US Department of Agriculture (USDA) Agriculture Research Service (ARS) centers to produce and characterize glandless cottonseed protein concentrates and isolates (70% and 90% protein, respectively).

"We are looking at the functional properties with the thought that the concentrates and isolates may have some unique applications," explains Michael Dowd, a chemical engineer at the USDA-ARS Southern Regional Research Center in New Orleans, Louisiana.

Dowd is collaborating with Mila P. Hojilla-Evangelista, a research chemist with the Plant Polymer Research Unit of the USDA-ARS National Center for Agricultural Utilization Research in Peoria, Illinois. They are comparing some of the chemical and functional properties of the protein isolates Dowd has produced from both glanded and glandless cottonseed.

"In my laboratory, we determined the protein samples' solubility in aqueous media at various pH, foaming properties, emulsification properties, water absorption, and sensitivity to heating," explains Hojilla-Evangelista. Both protein isolates (glanded and glandless) have similar solubility profiles (the graph of percent soluble protein vs. pH) and are "strikingly most soluble at very acidic pH," she notes. "When we analyzed the other properties, we did so at this acidic condition (pH 2) because this is where we detected the greatest amounts of soluble cottonseed protein. The emulsification properties and water absorption were again similar; both protein isolates were excellent emulsifiers and formed moderately stable emulsions. The foaming properties are where we observed notable differences: Glandless cottonseed protein isolate (CPI) had higher foaming capacity and far more stable foams than the protein from glanded cottonseed."

When Hojilla-Evangelista compared the CPI to soybean protein isolates (SPI)—based on work published in *JAOCS* (*81*:1153–1157, 2004)—she found that the CPI are markedly more soluble than SPI at pH 2, so the CPI have a potential market in applications with acidic pH. "CPI also appear to be better emulsifiers than SPI, and foaming capacity and stability of the glandless CPI are comparable to those of SPI," she said. "The CPI then could be viable alternatives to SPI for foaming and emulsifying uses."

Given these characteristics, Hojilla-Evangelista suggested a broad range of product development possibilities for CPI. The need for high solubility in very low pH applications would include high-protein fruit juices, sports drinks, possibly carbonated drinks, or tomato-based pasta sauces. CPI's foaming properties could lead to its use in whipped toppings, desserts, or baked products. Its emulsifying ability would be useful for sausages and similar comminuted meat products, confectionery products, some dairy products (ice cream, desserts, processed cheese), and some sauces or soups.

TABLE 1. Oil content of cotton seed[a]

Year	Nontransgenic Coker 312	Transgenic
2009	25.9%	27.3%
2010	24.9%	26.5%
2011	26.9%	29.2%

[a] Source: Keerti S. Rathore

"The first step entails transfer and stable integration of the transgene into the plant genome," he writes. "The second step involves the recovery of a transgenic plant from the stably transformed cell." In light of the difficulties involved in transforming cotton, Rathore and his team focused on *Agrobacterium*-mediated transformation of cotton because it does not require specialized equipment, is relatively inexpensive, and is more likely to result in single-copy transgenic events.

Rathore reports that the team thought at first that antisense technology would work. "The idea was to silence the gene that codes for the enzyme involved in the biosynthesis of gossypol—δ-cadinene synthase—in a seed-specific manner. We didn't want to mess with gossypol and related terpenoids in other parts of the plant in order to keep their protective qualities."

He and his team did manage to generate a number of transgenic cotton plants with the correct silencing construct that exhibited low gossypol content in the seeds and normal levels elsewhere.

"Things looked promising in the first generation," he says. "However, most of the low-gossypol lines lost the trait in the next generation." And so the TAMU researchers set the project aside in 2001–2002 because of lack of funding, even though they wanted to see if the new technique of RNA interference (RNAi) could solve the problem of heritability.

Work began again around 2003 using RNAi technology, and toward the end of 2005, the group had their first glimpse of a positive result. They saw some lines with ultra-low gossypol levels in the seed and confirmed the trait through to the second and third generations.

"The important thing was that the aerial parts and roots had the same levels of protective gossypol and related terpenoids as the parent plant," notes Rathore.

Andrew Fire and Craig C. Mello shared the 2006 Nobel Prize in Physiology or Medicine for their work on RNAi in the worm *Caenorhabditis elegans*, which they had published in 1998. The technique introduces small strands of synthetic ribonucleic acid (RNA) into cells to induce suppression of specific genes.

The TAMU team's RNAi work in cotton appeared in 2006 in the *Proceedings of the National Academies of Science* (*103*:18054–18059), detailing the results from three lines of transgenic plants grown in the greenhouse that showed seed specificity for the gene silencing.

"Since then," Rathore says, "we have done quite a bit more work on a couple of these lines. We wanted to address stability,

CONTINUED ON NEXT PAGE

Keerti Rathore in his laboratory at Texas A&M University. (Texas AgriLife Research photo by Kathleen Phillips)

so we took nine different lines through five generations in the greenhouse and found that the trait was stable."

Rathore notes that the seed-specific silencing occurs because of the DNA sequence of the promoter used, which his team isolated from cotton itself. Loosely, a promoter is localized before the coding DNA sequence for the actual protein that, when transcribed, produces an enzyme or other protein.

"The promoter determines whether the gene is expressed in the seed, root, or elsewhere," he says. "If we didn't have this promoter, which normally controls the gene for the seed protein α-globulin in cotton, we probably would not have had this success. The seed promoter and RNAi got the result."

Along with the stability testing, the group also tested young seedlings to see if they were capable of launching a gossypol-mediated defense response when challenged with a mold pathogen (*Rhizoctonia solani*). The seedlings were able to do so, and that work was published in 2012 in *Plant Biotechnology Journal* (*10*:174–183).

Two RNAi lines were tested for three years (2009–2011) in the field and were stable under field conditions, without any loss of fiber or seed quantity and quality (*Plant Biotechnology Journal*:doi:10.1111/pbi.12013, 2013). Analysis of the seeds by a private lab found them to be largely similar to nontransgenic seeds.

"With the exception of the ultra-low level of gossypol—at 200–250 parts per million (ppm)—the other constituents are similar, with one significant difference," says Rathore.

That difference bodes well for eventual commercialization: The team has seen consistently higher levels of oil in the transgenic seeds, and statistical analysis (not included in Table 1) shows the differences are significant.

"This makes sense," he says, "Gossypol constitutes almost 1% of the seed by weight. By knocking that back by 98–99%, resources apparently are diverted back into making oil."

NEXT STEPS

Rathore and Cotton Incorporated are committed to pushing the project forward. Toward that end, Rathore hopes to conduct multi-location field trials and, eventually, feeding studies. Another question that needs work is whether the gene construct can be moved into a commercial variety (most transformation work in cotton is done in a very old, noncommercial variety known as Coker 312). Further, researchers at TAMU in Corpus Christi are investigating using meal from the transgenic lines for aquaculture, particularly in shrimp.

Which raises an interesting point about protein from cottonseed meal. As it stands, the only mammals benefitting from cottonseed protein—either as whole seed or as meal after oil extraction—are cows, since only ruminants can tolerate gossypol.

Processing and marketing advantages

The potential advantages to oil mills of processing glandless cottonseed (and, by extension, presumably ultra-low-gossypol transgenic cotton) are many, according to work done several decades ago by researchers at Texas A&M University (TAMU) and the Anderson Clayton Co. (Houston, Texas, USA).

Some of the estimated advantages cited by E. W. Lusas and G.M. Jividen at the 1985 AOCS World Conference on Emerging Technologies in the Fats and Oil Industry follow. Additional testing at industrial scale would be required to verify these findings.

The proposed advantages include:

- The ability to hold crude cottonseed oil for three months or longer without the setting of color from gossypol (which turns raw cottonseed oil dark red or even black), thus eliminating the need for miscella or on-site conventional refining;
- A 3% reduction in loss of glandless seed oil in refining, with reduced requirements for alkali;
- A reduction in bleaching earth requirements of up to 50% (to a level of 0.5% earth used);
- The production of a light-colored oil, which is more competitive in the marketplace; and
- An expansion of markets and development opportunities for cottonseed meals.

Cottonseed kernels from Keerti S. Rathore's laboratory have been sliced through the middle to expose the gossypol-containing glands. On the left are nontransgenic Coker 312 parental seed kernels, showing multiple gossypol glands. On the right are seed kernels from Rathore's ultra-low-gossypol cottonseed (ULGCS) line 66–303, in which the gossypol level has been substantially reduced (>98%). Note that the faintly colored glands are still present in the ULGCS seeds.

"Cows are not the most efficient animals at converting feed to animal protein," notes Rathore. "It takes 5.8 pounds of feed to produce a pound of beef. Pigs are more efficient, with a feed-conversion ratio of 3.3 and chickens even more so at 2.1; some fish have a conversion factor that is close to 1. If you have a limited supply of feed, it makes more sense to feed it to chickens or fish than to cows."

What is needed now is a commercial partner that will move the trait into an established variety or varieties. "The big three seed companies have all indicated that they agree this technology will provide added value for growers and ginners, but they haven't figured out how they can capture enough value to make it worth their while," explains Wedegaertner.

The big three seed companies in cotton are Bayer CropScience, Dow AgroSciences, and Monsanto. Bayer is working with researchers in New Mexico (see sidebar), where insect pressure on cotton is less than elsewhere, on traditionally bred glandless cotton. Spokespersons for both Monsanto and Dow confirmed that glandless or low-gossypol cotton varieties are not in their product pipelines at this time. Based on recent publications, work continues in Australia and China—the world's No. 1 cotton producer—on transgenic low-gossypol cotton.

"Key to all of this," says Wedegaertner, "is the regulatory registration process for the TAMU transgenic cotton. It is clear from laboratory tests that what the TAMU team is doing with RNAi technology is simply mimicking what naturally occurs in mutant varieties. That is the reason we think regulatory approval will not be as time-consuming or expensive as with some other transgenic crops. And once it is registered, the seed companies may show more interest."

Wedegaertner is nothing if not optimistic about bioengineered ultra-low-gossypol cottonseed: "I expect it to be commercialized during my lifetime," he says, firmly. Just don't ask him, as jokesters sometimes do, how long he plans to live.

Catherine Watkins is associate editor of Inform *and can be reached at cwatkins@aocs.org.*

Making sense of e-sensing

Laura Cassiday

- Why use e-sensing?
- What are the advantages and limitations of traditional sensory panels?
- What are the applications of artificial noses, tongues, and eyes?

What makes your favorite chocolate chip cookie so delicious? Is it the comforting aroma, the sweet flavor of melting chocolate, the crumbly texture, or the ideal scattering of chocolate chips? Most people cannot define the precise qualities that make their favorite foods so enjoyable, so companies are increasingly turning to electronic sensing, or e-sensing, not only to improve the appeal of their products but also to optimize production methods, ensure quality and batch-to-batch consistency, and assess shelf life.

E-sensing most commonly refers to machines that mimic the human nose, tongue, or eye. Unlike their human counterparts, e-sensors provide unbiased, quantitative information about product attributes. Yet challenges remain if e-sensors are to be widely adopted by the food industry; and for some applications, only the human nose (or tongue or eye) knows.

TRADITIONAL SENSORY PANELS

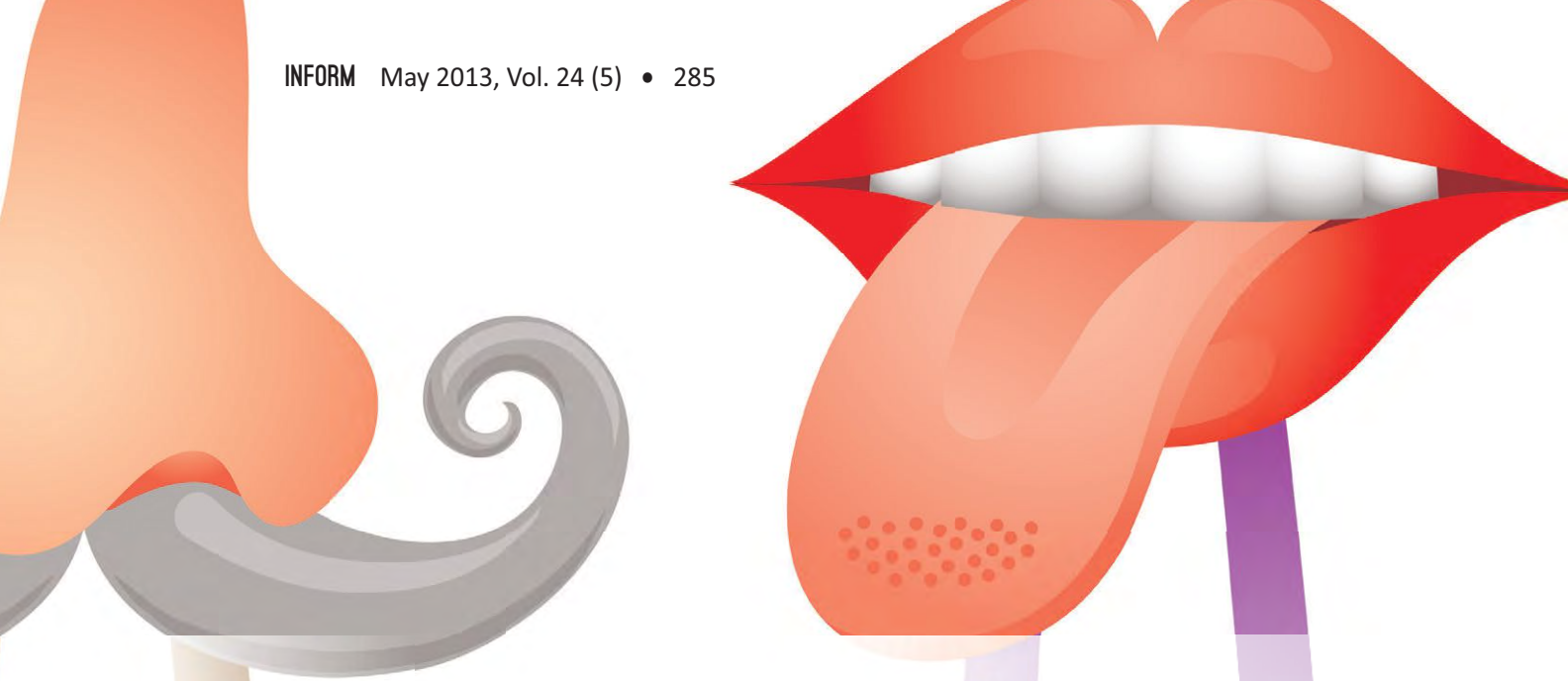
The food industry has long relied on sensory panels—groups of specially trained food testers—to provide useful information on a product's aroma, flavor, and visual appeal. Professional food testers, often gifted with naturally discriminating palates, must undergo extensive training that enables them to identify and then articulate specific characteristics of foods, such as a "grassy green" flavor or a "beany fried" smell. The information they provide can help manufacturers fine-tune their products to broaden customer appeal.

Despite being a mainstay of the food industry, sensory panels are not without their pitfalls. Even the most conscientious tester can harbor food biases that color her assessment of a product. Furthermore, a tester's physical condition (for example, having a cold) or emotional state can influence his perceptions. And although sensory panelists can rate the intensity of a smell or taste on a scale of 1 to 10, variability from person to person, or even from the same person on different days, limits the quantitative value of such information.

Another problem with sensory panels is that the human senses can become saturated with overuse. "The fatigue factor is a real killer," says John Shea, vice president of sales and marketing at Alpha MOS, a leading manufacturer of e-sensing devices with worldwide headquarters in Toulouse, France. "We work with a company that makes hot pepper sauce. After a few slugs of that stuff, the panelists' taste receptors are just shot."

The logistics of assembling a statistically meaningful number of trained panelists can also be difficult. "It's hard enough to find a partner to play tennis, much less bring 12 people in to do a sensory panel at the same time, with nobody suffering from a cold or taking any medications," says Michel Aliani, director of the Weston Sensory and Food Research Centre at the University of Manitoba, Winnipeg, Canada. In addition, ethical issues or general unpleasantness can limit the use of sensory panels for some applications, such as assessing the bitterness of pharmaceuticals or the smelliness of rancid oils.

For these reasons, many researchers and manufacturers are looking to supplement, if not replace, sensory panel data with



e-sensing. Fortunately, increasingly sophisticated e-sensors are commercially available. Alpha MOS, which introduced the first commercial artificial nose back in 1995, now offers three versions of odor analyzers for applications in the food, pharmaceutical, cosmetic, and automobile industries, as well as in areas as diverse as environmental monitoring and counterterrorism.

METAL OXIDE SEMICONDUCTOR-BASED NOSES

Alpha MOS's GEMINI and FOX e-noses operate on principles similar to the human nose. When a person inhales, airborne odor molecules enter the olfactory epithelium, a mucus-covered tissue in the roof of the nasal cavity. There, the odorants encounter about 1,000 different types of olfactory receptor proteins on the surfaces of neurons. The binding of odor molecules to receptors triggers nerve signaling, which the brain decodes as a particular odor. Each receptor can bind to several different odor molecules with varying intensities. Therefore, it is the complex pattern of receptor responses, rather than the response from any single receptor, that identifies the odor. This receptor cross-reactivity greatly expands the number of odors that the nose can detect.

Similar to the human nose, the GEMINI and FOX e-noses contain arrays of sensors that respond to odor molecules. But instead of proteins on the surfaces of cells, the e-nose sensors are metal oxide semiconductors with slightly different compositions and reactivities. When volatile compounds adsorb on the surfaces of the sensors, they cause a change in electrical resistance that the machine detects. Like human olfactory receptors, each of the metal oxide sensors contained in the GEMINI (6 sensors) and the FOX (18 sensors) models cross-reacts with multiple odor molecules at different intensities. The sensitivity of the FOX e-nose to most odors is equal to or better than that of the human nose—typically in the low parts-per-million (ppm) range.

To analyze the odors of a sample, researchers add the sample to a vial and place it in the instrument. The vial is heated, then the e-nose analyzes the volatile compounds in the headspace (i.e., the space in the vial above the sample). The odors in the sample produce a unique pattern of sensor reactivity, or "fingerprint."

For quality control applications, workers at a manufacturing plant can train the e-nose to recognize "good" samples and flag "bad" ones. In the training phase, workers analyze at least six good samples as standards. The e-nose then assesses the variability in the standards and sets upper and lower warning limits. When future samples are analyzed, the system flags samples whose odor fingerprint falls outside the acceptable limits, ensuring batch-to-batch consistency (see graph of pizza sauce testing in the supplement to the digital edition. Log in to read the May 2013 issue at www.aocs.org/login.).

Aliani has used the FOX e-nose to monitor changes in cooking oil over time in commercial fryers. "Fast food restaurants would like to know how many days they can reheat oil in a commercial fryer without altering the smell of their French fries," says Aliani. So he and his coworkers used the e-nose to monitor changes in the odor fingerprint of French fries cooked in the same oil over a period of 15 days (unpublished data).

According to Shea, Alpha MOS scientists are collaborating with researchers at the Institute for Food Safety and Health at the Illinois Institute of Technology in Bedford Park, Illinois, USA, to assess the ability of the e-nose to detect adulterated olive oils. "Extra virgin olive oil is highly valued by consumers for its health benefits, but olive oil adulterated with lower-grade oils is making its way into the market," says Shea. Alpha MOS's e-nose may be able to detect not only the oil's adulteration with cheaper oils, but also the oil's region of origin, helping to prevent false labeling.

The e-nose can also help determine a product's shelf life and how changing ingredients or storage conditions affects shelf life. Furthermore, the system can detect gases that are released from packaging materials into foods, altering the odor and perhaps the safety of the product. Another application is in taste masking. "If you want to put some omega-3 oil in your cookies or your orange juice, the e-nose can help you select ingredients to mask the fishy smell," says Jean-Christophe Mifsud, founder and chief executive officer of Alpha MOS.

A GAS CHROMATOGRAPHY (GC) SNIFFER

In 2011, Alpha MOS expanded its e-nose portfolio by introducing the HERACLES ultra-fast GC system. It operates on an entirely different principle from the metal oxide sensors and offers

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FIG. 1. A colorimetric sensor array, which is smaller than a postage stamp, contains pigments that change color in response to their chemical environment. This e-nose can differentiate brands of Colombian coffee by the odors they produce, as seen by the different colored patterns. Credit: Kenneth Suslick.

expanded capabilities for odor analysis. "If you want just a firewall to detect rancidity or other quality control parameters, then the FOX or GEMINI system is definitely for you," says Mifsud. "But if you want to know why you have a flag and what types of molecules are involved, we would recommend the HERACLES."

GC separates volatile compounds and identifies them by how long it takes the compounds to traverse a column. The column is filled with a material that interacts with the analyzed compounds to varying degrees. Researchers can identify volatile compounds, including odors, by comparing their retention times, or the time it takes for them to exit the column, with reference compounds.

Standard GC for most odor molecules takes about 30–40 minutes per run. However, the HERACLES e-nose heats the separation column at a high rate, speeding up the analysis to as little as 40 seconds. The HERACLES rapidly identifies odors by comparing their Kovats Index (a measure of retention time that is independent of the particular GC system) to those in a large database of compounds. The database, called AroChemBase, contains 44,200 compounds, 1,900 of which have associated sensory attributes. Therefore, the HERACLES system can quickly identify molecules that differ between samples, and what type of smell they produce. The device is very sensitive, able to detect most odors at parts-per-billion (ppb) concentrations.

The HERACLES e-nose also includes software to convert data from multiple peaks in a gas chromatogram into a single point on a two-dimensional principal components analysis (PCA) map. This form of data visualization greatly simplifies the comparison of global odor profiles among samples.

According to Mifsud, the HERACLES e-nose is particularly helpful for competitor benchmarking. "You might want to know how to copy your competitor's product or what type of ingredients they're using," he says. For example, a salad dressing manufacturer wanted to mimic a competitor's dressing (Brand A). So it used the HERACLES e-nose to compare its best imitation with Brand A. The HERACLES identified the odor chemicals that differed between the two dressings and also provided important clues as to what ingredients to add to better imitate Brand A. "The system very rapidly analyzed the chances that Brand A had caraway, cumin, lemon, mint, and rosemary in its dressing," says Mifsud.

SMELLING IN COLOR

Other researchers have devised e-noses that operate on different principles, with their own target applications. Kenneth Suslick, professor of chemistry at the University of Illinois at Urbana-Champaign, developed an e-nose that identifies odors by the color changes they cause in pigmented substances.

Suslick's e-nose consists of an array of 36 chemically responsive dyes printed onto a piece of plastic the size of a postage stamp. Suslick and his colleagues place the array in a chamber with the sample to be analyzed. Volatile compounds react with the dyes, changing their colors. Importantly, the e-nose is quantitative: The degree of color change reflects the concentration of the odor. A simple camera, similar to the kind found in cell phones, takes a picture of the array. The system then compares the color profile to that of previous samples to obtain a match.

According to Suslick, the colorimetric device overcomes some of the limitations of previous e-noses. For example, metal oxide sensors deteriorate with time and need to be recalibrated frequently. However, the colorimetric sensors are essentially disposable: Because they are simple and inexpensive to make, the arrays can be used once or a few times and then thrown away. In addition, the dye arrays are unaffected by changes in humidity, which can cause major problems for metal oxide arrays.

The colorimetric e-nose is also more sensitive and selective than metal oxide-based e-noses, Suslick says. "Previous sensors reacted only weakly with odor molecules," he says. "Our approach was to look at the olfactory system itself, which uses much stronger interactions." Suslick's team noticed that roughly three-quarters of all olfactory receptor sequences contain a highly conserved metal ion-binding site. Suslick hypothesized that olfactory receptors bind strongly to odor molecules through coordination of a metal ion.

Similarly, metal ions in metalloporphyrin dyes bind to various chemicals, changing the colors of the dyes. Suslick decided to use these dyes, as well as others that are sensitive to the pH, polarity, or oxidation-reduction activity of an odor chemical, as the detection system for his e-nose. The result was a sensitive e-nose that can detect odor molecules at ppm to ppb concentrations.

The e-nose has proven effective for a variety of purposes, including the discrimination of different brands of coffee, beer, and soft drinks, and the detection of toxic industrial gases and the explosive used in shoe bombs. The e-nose may also have medical applications. Suslick and his coworkers have shown that the device can rapidly identify pathogenic bacteria from the volatile compounds they produce. In addition, the e-nose can detect early-stage lung cancer from a person's breath. Cancer cells produce different metabolic by-products than normal cells, some of which are volatile and detectable by the e-nose. Suslick co-founded a company called Metabolomx (Mountain View, California, USA) to commercialize the cancer-sniffing device. He expects a handheld version of the e-nose to be available in late 2013.

Suslick says that the e-nose cannot identify individual odors in a complex mixture, but he does not see that as a problem for most applications (Fig. 1). "You really don't want to know what 978 different compounds are in that cup of coffee you're drinking," he says. "What you want to know is, is this pure Colombian coffee, or has it been adulterated? Were the beans roasted properly, or were they overroasted? Normally you want a comparison to a standard rather than a component-by-component analysis, and our device is very good at that."

ARTIFICIAL TONGUES

Several versions of e-tongues also have been developed. These devices mimic the ability of the human tongue to detect the five basic tastes of sweet, sour, bitter, salty, and umami. Taste receptor cells, which are nerve cells clustered in the taste buds, detect salty and sour tastes by the passage of sodium ions or hydrogen ions, respectively, through channels in their membranes. Sweet, bitter, and umami tastes are registered by the binding of molecules to taste receptors on the cell surface.

Alpha MOS launched the ASTREE electronic tongue in 2000. The device contains seven cross-reactive sensors with organic membranes that interact with ions and chemical compounds. Researchers can analyze liquids, like beverages, directly, whereas solid samples such as foods or pharmaceuticals must be dissolved in a liquid. Workers dip the sensors into the liquid sample and measure the potentiometric difference between each sensor and a reference electrode. The pattern of sensor responses generates a taste fingerprint for the sample. PCA analysis allows researchers to visualize the taste qualities of a sample as a single point on a two-dimensional map. For example, different brands of potato chips can be plotted as a function of their acidity and saltiness.

For even more powerful sensory analysis, researchers can combine data from the electronic nose and electronic tongue on a single PCA map. The results correlate well with data from human sensory panels, Mifsud says. The combination of the FOX e-nose and ASTREE e-tongue discriminated as well as a sensory panel among several brands of potato chips (see graph of sensory attributes in the supplement to the digital edition. Log in to read the May 2013 issue at www.aocs.org/login.).

In 1993, Kiyoshi Toko, professor of information science and electrical engineering at Kyushu University, Fukuoka, Japan, developed a taste sensor based on artificial lipid membranes. An

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TABLE 1. Chemical components of the Insent taste sensora

Taste sensor	Artificial lipid	Plasticizer
Saltiness sensor CT0	Tetradodecylammonium bromide 1-Hexadecanol	Dioctyl phenylphosphonate
Sourness sensor CA0	Phosphoric acid di(2-ethylhexyl) ester Oleic acid Trioctylmethylammonium chloride	Dioctyl phenylphosphonate
Umami sensor AAE	Trioctylmethylammonium chloride	Dioctyl phenylphosphonate
Bitterness sensor C00 (for acidic bitter materials)	Tetradodecylammonium bromide	2-Nitrophenyl octyl ether
Bitterness sensor BT0 (for bitter hydrochloride salts)	Phosphoric acid di-*n*-decyl ester	Bis(1-butylpentyl) adipate Tributyl *O*-acetylcitrate
Bitterness sensor AN0 (for basic bitter materials)	Phosphoric acid di-*n*-decyl ester	Dioctyl phenylphosphonate
Sweetness sensor GL0 (prototype)	Tetradodecylammonium bromide Gallic acid	Dioctyl phenylphosphonate

[a]Credit: Kiyoshi Toko

improved version of the sensor is now marketed by the Japanese company Intelligent Sensor Technology, Inc. (Insent). Toko and his colleagues refer to the Insent device as a "taste sensor" to differentiate it from electronic tongues offered by Alpha MOS and other companies, which operate on a different principle.

Toko's taste sensor makes use of lipid molecules with different hydrophobic and hydrophilic groups, such that they interact electrostatically and hydrophobically with taste compounds. The desired lipids and a plasticizer are assembled into a membrane and then attached to an electrode sensor (Table 1). After immersing the sensor into a tasteless reference solution, researchers place the sensor in the sample solution and measure the membrane potential that results when taste substances interact with the lipids.

The device incorporates up to eight sensors, which vary in lipid composition so that each is sensitive to different tastes (Fig. 2, page 335). For example, the saltiness sensor contains a hydrophilic lipid membrane because salty substances in solution form ions that interact electrostatically with the membrane. In contrast, bitterness sensors are hydrophobic because most bitter compounds are hydrophobic and therefore adsorb onto the membrane.

In contrast to most e-tongues, the taste sensor requires no complex statistical methods, such as PCA, for data interpretation. Each of the taste sensors is selective for a specific taste, with little or no cross-reactivity, which greatly simplifies data interpretation. Data from the eight sensors can be converted into 11 types of taste information: saltiness, sourness, umami, richness, bitterness, astringency, aftertaste from three kinds of bitterness, aftertaste from astringency, and sweetness. In addition, the device is quantitative and can discriminate both taste quality and intensity between samples.

Toko has demonstrated the taste sensor's effectiveness in differentiating brands of green tea, beer, and Prosciutto ham (see radar charts in the supplement to the digital edition. Log in to read the May 2013 issue at www.aocs.org/login.). For quality control purposes, the sensor has been used to monitor changes in taste qualities of green tea with aging and with different lots. The coffee distributed by Japan Airlines was perfected using Toko's taste sensor. "If humans try to develop coffee, they need many trials and errors of tasting it," he says. "However, if the target taste is determined, creation of the desired taste is very easy using the taste sensor."

The taste sensor has also provided useful information on how taste substances interact with each other. Toko and coworkers showed that an edible oil selectively suppresses the bitterness and astringency of a sample, making foods taste milder. Also, the researchers used the taste sensor to identify the best compound for minimizing the bitterness of the antimalarial drug quinine hydrochloride. Toko notes that an independent research group found that, for the evaluation of pharmaceutical formulations, the Insent taste sensor showed high correlation with a human sensory panel and excellent day-to-day reproducibility (Woertz *et al.,* 2011).

THE EYES HAVE IT

Recent innovations in digital imaging have enabled the development of sensitive electronic eyes. Alpha MOS's IRIS electronic eye is a high-resolution CCD [charge-coupled device] camera combined with powerful data processing software. Researchers place one or more samples in the device's 600 × 600 × 750-mm closable light chamber, and the CCD camera takes a picture. The data processing software extracts color and shape parameters from the picture and can then correlate these data with data from sensory panels to determine the precise visual characteristics that consumers prefer.

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BRIEFS

The Cocoa Research Institute of Ghana will have a new flavor laboratory, including a trained sensory panel, thanks to a joint effort by the World Cocoa Foundation (WCF; Washington, DC, USA) and luxury chocolate maker TCHO (San Francisco, California, USA). Further, an outreach program will allow farmers to taste chocolate from their own beans and learn how product quality depends on good fermentation and drying methods, according to a statement by WCF.

■■■

Farmer-owned energy, grains, and foods company CHS Inc. (Inver Grove Heights, Minnesota, USA) recently announced a 50:50 joint venture (JV), known as CHS AGRO, with South American agriculture company Adecoagro. The new JV, based near Buenos Aires, Argentina, will grow and process sunflower kernel and in-shell products for global distribution.

■■■

A teenager created an online petition that was signed by 200,000 people to push PepsiCo Inc. (Purchase, New York, USA) to remove brominated vegetable oil (BVO) from its line of Gatorade sports drinks. The teen, Sarah Kavanagh of Hattiesburg, Mississippi, USA, read the fine print on a Gatorade bottle and saw BVO—an emulsifier first used in 1931 that is still used in a number of citrus-flavored soft drinks—on the list. After researching BVO online, she found that brominated compounds (including BVO) have been banned in Europe, Japan, and India because they are suspected of being endocrine disruptors.

Pepsico said it will remove the BVO in Gatorade by the end of the second quarter of 2013 and replace it with sucrose acetate isobutyrate. The company added that the decision was not based on Kavanagh's petition. Further, BVO will remain in several other soft drinks manufactured by PepsiCo, including Mountain Dew. About 10% of soft drinks sold in the United States contain BVO, according to *The New York Times* newspaper. ■

NEWS & NOTEWORTHY

FDA releases food traceability report under FSMA

Stakeholders had until April 4, 2013, to submit comments to the US Food and Drug Administration (FDA) on recommendations contained in a report FDA released on March 4 on two pilot projects on food product traceability that were conducted by the Institute of Food Technologists (IFT; Chicago, Illinois, USA).

The pilot projects were mandated by the Food Safety Modernization Act, which was signed into law in January 2011 but has yet to be fully implemented. FDA eventually will release its own report to the US Congress on food tracking and tracing together with its final recommendations on traceability. At press time, FDA had not indicated when it will publish the FSMA regulations on imported food/traceability and animal feed; the agency released its regulations on preventive controls and produce safety in January 2013. The comment period on those rules ends on May 16, 2013.

IFT, under contract to FDA, conducted two product tracing pilot studies of foods (including ingredients) that had been implicated in foodborne illness outbreaks between 2005 and 2010. One pilot focused on the tracing of chicken, peanuts, and spices in processed foods; the other focused on the tracing of tomatoes. The aim of the pilot studies was to assess the costs and benefits of tracking the designated foods as well as to determine the feasibility of methodologies and technologies currently in use by the food industry.

The report suggests that FDA should:

- Establish uniform recordkeeping requirements for all FDA-regulated foods and not permit exceptions based on risk classification;
- Identify and maintain CTE (Critical Tracking Events) and KDE (Key Data Elements), as determined by FDA;
- Require industry to develop, document, and exercise a product tracing plan;
- Support industry-led initiatives for the development of implementation guidelines;
- Communicate more clearly about information the agency needs during tracing investigations;

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- Develop standardized electronic reporting mechanisms;
- Accept CTE and KDE in summary form to expedite investigations;
- Request CTE and KDE data for more than one step up and one step down in the supply chain;
- Use technology to share and analyze data reported and to make the technology platform available to other regulatory entities; and
- Coordinate investigations with state and local agencies using industry subject matter experts as appropriate.

For the full IFT report, see http://tinyurl.com/IFTreport.

Transforming the food and agriculture supply chain

Rabobank—the agricultural banking cooperative based in Utrecht, Netherlands—has published a new report looking at the current food and agriculture (F&A) supply chain, identifying flaws that leave the sector ill equipped to respond to new complexities and calling on the industry to transform the way supply chains are organized. Specifically, Rabobank identifies a dedicated supply chain model as the best one for F&A companies to implement, and recommends the adoption of longer-term supply agreements and cooperative relationships with upstream and downstream partners. (In a dedicated supply chain structure, upstream suppliers and processors enter into long-term partnerships with each other and a downstream chain leader. Crucially, information and insights are shared along the chain's length for the benefit of all members.)

In the report, which was written by Rabobank's global Food & Agribusiness Research and Advisory unit, the bank's analysts look at how the operating environment for F&A companies is becoming increasingly complex, as new external influences compound traditional pressures such as rising commodity prices. Rabobank says that the dedicated supply chain model has potential to revolutionize the F&A industry by making it more productive, innovative, safe, and sustainable. The report stresses that all of these outcomes are vital if the sector is to deliver food security to a future global population of 9 billion.

LIMITATIONS OF THE CURRENT STRUCTURE

Rabobank says that traditional pressures on the F&A industry (supply and demand dynamics, a burgeoning population, and rising commodity prices) are being compounded by a new set of external influences. The direct use of commodities for biofuel production and an increased awareness of the energy intensity of food production, for example, have embroiled F&A companies in an ongoing food vs. fuel debate. Similarly, speculation in commodity markets and the regulatory responses from governments that this has triggered have added to the complexity of the environment in which the F&A sector operates.

These new pressures exacerbate the flaws in the current supply chain model, Rabobank says. The dominant supply chain model is currently structured in a linear fashion, in which suppliers, processors, and retailers form short-term partnerships independent from the influence and interests of other members of the chain. This model is highly inefficient, restricting F&A companies' ability to respond to changes in supply and demand dynamics, while fleeting partnerships limit productivity and restrict innovation. This system also results in wasteful processes that cause more environmental degradation than is necessary.

ADDING VALUE THROUGH CLOSER COOPERATION

Rabobank believes that switching to the dedicated supply chain has the potential to transform the F&A industry. Justin Sherrard, Rabobank global strategist, says, "Closer cooperation of this sort will transform the nature of F&A partnerships from transactional ones that are centered around chasing price, to a system focused on creating value."

The advantages of dedicated supply chains over the current system are many, Rabobank says. Companies embracing this thinking will benefit in the following ways:

- *Reduced risk.* Longer-term, more stable agreements reduce exposure to price volatility, whereas shared insights will enable players to react better to market risks.
- *Improved productivity.* Better insights into chain requirements improve process efficiency, and partners can also work together to finds ways to limit or reuse waste.
- *Access to new markets.* Better insights into downstream needs and opportunities can better inform product innovation and help companies to develop new markets.
- *Enhanced brand and reputation.* Companies with ambitious corporate social responsibility targets can help their partners on other product attributes, such as sustainability.

Rabobank says that adopting the dedicated supply chain model positions F&A companies for longer-term growth, as the sector rises to meet the overarching challenge to feed the world in coming decades.

MAKING THE CHANGE

The Rabobank report calls for prominent F&A companies to show leadership by creating initiatives that will lead to closer cooperation among their upstream partners. The report cites several examples of leading F&A companies that are already active in this area, such as Mars' decision to release the cacao genome sequence into the public domain as part of its broader commitment to sustainably sourcing all cocoa purchases by 2020.

Canola still top Canadian revenue-producing crop

Farm gate receipts for canola deliveries in 2012 were $8.1 billion in Canada, according to estimates in Agriculture and Agri-Food Canada's (AAFC) Farm Income Forecast, which was released at the end of February 2013.

That figure is 5.4% higher than the final Statistics Canada tally of $7.7 billion in farm gate receipts for canola in 2011. Wheat generated $6.3 billion in farm gate receipts in 2012. AAFC forecast total farm cash receipts for all crops at a record $29.0 billion for 2012, a 12% increase over 2011. Total farm cash receipts for all livestock are estimated at $21.1 billion, a 4% increase over 2011. ■

SUSTAINABILITY WATCH

Sustainability in Brazil

There is growing interest in the idea of "green" behavior and products in Brazil, according to market research firm Mintel.

Indeed, fully 73% of Brazilian consumers claim that their individual behavior can "really have an effect" on the world's environment, the company said recently in a statement about its *Green Lifestyles—Brazil* report, which was published in January 2013.

Despite this claim, Mintel found that Brazilians are less likely to consider environmental factors if doing so will cost more money, with 69% claiming that prices concern them more than the environment. In addition, some 73% of consumers claim that "the main reason I reduce water or electricity consumption is to save money." Furthermore, some 91% said that energy-efficient products save money in the long run, even if they cost more to buy.

Sheila Salina, research analyst at Mintel Brazil, said: "Even when they show great concern for the environment, it seems Brazilian consumers are not prepared to accept solutions that will cost extra. Consumers are constantly bombarded with information [about] sustainability, increasing confusion, which makes consumers more skeptical about the claim. The most successful way to address this is to combine affordability and functionality with the use of sustainable technologies. Companies and products that are capable of aligning price, quality, and sustainability will be best positioned to capture consumer attention and capitalize [on it] as a result."

It seems as if new product developers are also capitalizing on consumer demand in this area. Analysis from Mintel's Global New Products Database shows that a quarter (24%) of new introductions of consumer packaged goods in 2012 had either an ethical or an ecological claim. This percentage is almost identical to the proportion of products with ethical claims that were introduced in the United States (23%) in 2012, but lower than the proportion of introductions in the United Kingdom (31%). ■

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I hereby subscribe to the above Code of Ethics. Signature of Applicant _____

BRIEFS

Biofuel developer Piedmont Biofuels (Pittsboro, North Carolina, US) earned certification from the Roundtable on Sustainable Biofuels (RSB) program for excellence in biodiesel production in February 2013. The RSB is an international initiative coordinated by the Energy Center at the Swiss Federal Institute of Technology in Lausanne, Switzerland, concerned with ensuring the sustainability of biofuels production and processing.

SCS Global Services, an independent testing company, performed the certification by examining Piedmont's facilities. Piedmont qualified by producing biodiesel that reduces greenhouse gas emissions by 70% compared to conventional diesel fuel and deploying both active and passive solar equipment and strategies.

■■■

On March 8, 2013, KLM Royal Dutch Airlines and Boeing made the first of 26 weekly flights testing the efficiency and sustainability of several advanced technologies. A KLM Boeing 777-200 flew from Amsterdam to New York City, partially powered by second-generation biofuel made from processed frying fat. The biofuel is being supplied by Sky NRG, which KLM founded in 2009 with the North Sea Group and Spring Associates.

The flights are also testing new software designed to optimize the aircraft's speed and reduce noise and emissions. Based on the results of these flights, KLM and Boeing will establish new operational procedures and recommend further technological developments.

■■■

In late March 2013, two biodiesel plants using wastes from the processing of tilapia fish as feedstock opened in the Brazilian towns of Jaguaribara and Morad Nova, in the state of Ceará. The development of these plants came in response to contamination of groundwater resulting from dumping fish viscera, which represent

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BIOFUELS NEWS

Argentina, Brazil reaching limits of biodiesel growth

These countries may have reached the limit of their biodiesel industry growth, according to a Rabobank report (http://tinyurl.com/Biodiesel-Brazil-Argentina). Dwindling support for first-generation biofuels, biofuel mandates, and international policies are contributing factors.

Brazil develops biodiesel mostly for its own use, but higher costs from a cumbersome tax structure and logistical problems limit Brazilian international competition, according to the report. Additionally, the Brazilian biodiesel industry needs to improve its efficiency. To do this, companies need to invest in increasing crushing and oil refining capacity, consolidating, or developing alternative feedstocks.

Argentina's biofuel industry depends on exports, but because the European Union (EU) has accused Argentina of dumping biodiesel in its market, Rabobank indicates the country's future growth will rely on government policies regarding domestic market pricing and the competitiveness of soy-based biodiesel with respect to alternative feedstocks.

EU regulations with respect to the Renewable Energy Directive may also affect Argentina's biofuel exports. But according to the report, the country's industry is expected to remain stable, and it will not match the explosive growth of the past.

Sapphire moving ahead with algae

Commercial sales. Sapphire Energy, Inc., a California-based company developing systems to grow algae outdoors for use as feedstock for biodiesel, announced on March 20 that it had entered into a commercial agreement with Tesoro Refining and Marketing Co. Under the agreement, Tesoro will purchase two barrels, or 320 liters, per day of crude oil derived from algae biomass that is cultivated

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10% of the live weight of the fish, onto the ground. In the factory, 50% of the tilapia discards will be turned into grease. Biofuel production of about 8,000 liters per day is anticipated. Petrobras, the Brazilian energy corporation, is expected to be the primary purchaser of the fuel.

■■■

The US Environmental Protection Agency issued a final rule on March 5, 2013, identifying additional fuel pathways that it determined meet the biomass-based diesel, advanced biofuel, or cellulosic biofuel lifecycle greenhouse gas reduction requirements specified in the Clean Air Act section 211(0), the Renewable Fuel Standard Program, as amended by the Energy Independence and Security Act of 2007. This rule determined that biofuels produced from camelina oil feedstock are acceptable, thus providing the certainty necessary for investment to bring camelina oil into commercial production for this purpose (http://tinyurl.com/FedRegister-camelina).

■■■

With the return of spring temperatures, SeQuential-Pacific Biodiesel, with a production facility in Salem, Oregon, USA, resumed its sale of B99 (99% biodiesel, 1% petrodiesel) in its over 30 retail outlets located in the US states of Washington and Oregon. It offers B50 to its customers during November through February to avoid the possibility of gelling of higher-biodiesel-content fuel during the colder temperatures of winter. In March it was selling B99 for $3.99 per gallon ($1.05 per liter).

■■■

The Brazilian government announced its intention on March 1, 2013, to increase the amount of ethanol mixed into gasoline sold at retail pumps to 25% on May 1. The blend had been 20% since 2011. But on March 18, Bloomberg news service reported that Duarte Nogueira, a deputy who represents São Paulo state for the Brazilian Social Democracy Party, indicated that as many as 20% of the sugarcane mills in Brazil's center south may close or be sold during 2013 because they are selling the ethanol they produce from sugar at a loss (http://tinyurl.com/Brazil-ethanol). Nogueira indicated that raising the blend of ethanol in gasoline to 25% is insufficient to make the mills profitable. ■

and harvested at Sapphire's Green Crude Farm in Columbus, New Mexico, USA (http://tinyurl.com/GreenCrudePerspective).

Sapphire has developed processes to extract oil from wet algae without the necessity of drying them first, a procedure that many other algae-producing companies use despite its cost and time consumption. Company tests have shown that their Green Crude oil can be refined into diesel fuel meeting ASTM 975 specifications.

Tesoro's Vice President of Renewable Development Joel Larkin said in a company statement: "We are pleased to become a purchaser of Sapphire Energy's Green Crude, which shows promise as an alternative fuel solution." The price per barrel that Tesoro will pay Sapphiare has not been disclosed (http://tinyurl.com/GreenCrudePerspective).

Tesoro and its seven refineries process about 675,000 barrels (~100 million liters) of oil per day to supply to their 1,375 retail sites located in the western United States.

Research on yield limitation. Sapphire recently published results of their collaboration with scientists at the University of Alabama and San Diego State University to identify the morphology, ultrastructure, and life history of the algal parasite *Amoeboaphelidium protococcarum*. This organism was isolated from an outdoor pond, open to the air, in which the alga *Scenedesmus dimorphus* was being cultivated for use as a biofuel feedstock.

The researchers found that as the levels of the genomic DNA of the parasite increased, the concentration of *S. dimorphus* cells decreased. Transmission electron microscopy of the parasite revealed that, at maturity the unwalled parasite occupied the entire host cell.

Alex Arvanis, chief science officer at Sapphire Energy, said in a company statement, "Identifying and overcoming crop protection challenges . . . is critical to ensuring successful, scalable algae farming, This collaborative study lays the groundwork for the better understanding and creation of innovative strategies that will facilitate algae biofuel production at commercial scale." For further information see http://dx.plos.org/10.1371/journal.pone.0056232.

Solazyme moving forward

In a company statement released at the end of February 2013, Solazyme, Inc., a renewable oil and bioproducts company, reported progress in reaching its technology, production, and commercialization goals. Jonathan Wolfson, CEO of Solazyme (South San Francisco, California, USA) predicted that that company would be "producing our high-value tailored oils on three continents by 2014."

Solazyme is developing products based on oil produced during the fermentation of algae. For example, in 2012 Solazyme and Mitsui and Co., Ltd. agreed to develop jointly a suite of triglyceride oils for use primarily in the oleochemical industry. Solazyme is developing multiple high-value oils, including high-oleic oil, high-myristic oil, and cocoa butter substitute, and is cooperating with Archer-Daniels-Midland to produce oils in Clinton, Iowa, USA, and with Bunge in Brazil (*Inform 24*:22, 2013).

Propel Fuels, a retailer of renewable fuels, and Solazyme reported good results from their 30-day pilot program to sell algae-based biodiesel in a commercial market (*Inform 24*:22, 2013). The companies cooperated to sell the fuel in a B20 (20% biodiesel, 80% petrodiesel) blend at Propel's gas stations in the San Francisco Bay area. Sales at Propel stations offering the algal fuel exhibited a 35% volume increase over Propel stations that were not participating in the pilot. A consumer survey found that 92% of participants would be more likely to purchase algae-derived fuel for its environmental benefits, and nearly 40% said they would pay

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BRIEFS

Dietary antioxidants may not protect against stroke or dementia, according to a study in *Neurology* (doi:10.1212/WNL.0b013e3182840c84, 2013). Elizabeth E. Devore of the Harvard Medical School in Boston and the Erasmus Medical Center in Rotterdam, Netherlands, led the research, which involved 5,395 persons, aged 55+, who had no signs of dementia at the start of the study. Of the participants, almost 600 subjects developed dementia during a median 13.8-year follow-up period and about 600 participants had a stroke. The researchers found that persons with high intakes of antioxidants were no more or less likely to develop brain disease than people with low intakes of antioxidants. This study differs from previous research that found that higher levels of dietary antioxidants were associated with a lower risk of stroke, the researchers noted.

■■■

Finnish researchers examined the impact of diet on the gut microbiota in identical twins. They found that intakes of energy, monounsaturated fatty acids, omega-3 polyunsaturated fatty acids (PUFA), omega-6 PUFA, and soluble fiber had significant associations with the amount of bacteria in the stool. In addition, co-twins (each set of twins) with identical energy intake had more similar amounts and diversities of bacterial species than did the co-twins with different intake. The co-twins who ingested the same amounts of saturated fatty acids had very similar bacterial profiles, whereas the co-twins with similar consumption of fiber had a very low profile similarity. "In conclusion, our findings confirm that the diet plays an important role in the modulation of the stool microbiota," according to the researchers, who were led by Catarina D. Simões of the VTT Technical Research Centre of Finland in Espoo. Their work appeared in *The Journal of Nutrition* (*143*:417–423, 2013). ■

HEALTH & NUTRITION

First clinical trial of the Mediterranean diet reported

Observational and epidemiological studies have pointed to the possible health benefits of a Mediterranean diet rich in olive oil, nuts, fish, legumes, vegetables, and fruits. Now, the first major multicenter clinical trial to examine the effect of a Mediterranean diet on a population at risk for heart disease has ended early, after almost five years, because the outcomes were so clear that continuing the trial would have been unethical.

The magnitude of the results surprised both the researchers and experts asked to comment on the study by the many media outlets covering the story: Those subjects following a standard Mediterranean-style diet with either a daily serving of a fistful of nuts or four tablespoons (~53 grams) of extra virgin olive oil (EVOO) had a 30% lower risk of a stroke, heart attack, or death from heart disease than those following the control diet.

The study, which was led by Ramon Estruch of the Hospital Clinic in Barcelona, enrolled almost 7,500 subjects (57% women) at risk of heart disease but without cardiovascular disease at the time of enrollment. Most participants were taking medication for high cholesterol, high blood pressure, or diabetes; they did not alter their regimen during the study.

Participants in the two Mediterranean-diet groups were given, gratis, either EVOO (from Spanish producers) or 30 grams (g) of mixed nuts/day (15 g of English walnuts, 7.5 g of hazelnuts, and 7.5 g of almonds) at no cost. Members of the control group received small nonfood gifts and were told to reduce their dietary fat intake. In particular, they were told not to eat nuts, red meat, or oils and oil-based dressings. Despite those instructions, the control group—called the "low-fat group" in the study—had fat intake

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that was almost identical to the groups consuming EVOO and nuts. The mean total fat consumption (as % energy) at the end of the trial was 41.2% for the EVOO group, 41.5% for the nut group, and 37.0% for the control group.

"Participants in the two Mediterranean-diet groups significantly increased weekly servings of fish (by 0.3 servings) and legumes (by 0.4 servings) in comparison with those in the control group," the study authors noted. In addition, participants assigned to a Mediterranean diet with EVOO and those assigned to a Mediterranean diet with nuts significantly increased their consumption of EVOO (to 50 g and 32 g per day, respectively) and nuts (to 0.9 and 6 servings per week, respectively)."

The researchers assessed compliance—which was high—through periodic measurement of hydroxytyrosol (a phenol present in EVOO) in urine and of α-linolenic acid (a marker of nut consumption) in blood. They tracked a combination of strokes, heart attacks, or heart-related deaths as the primary end point. There were 96 of these events in the EVOO group, 83 in the nut group, and 109 in the "low-fat" group.

Estruch told *The New York Times* that he felt the results were due to the "entire package" and not just the EVOO or nuts. His study appeared in *The New England Journal of Medicine* (http://www.nejm.org/doi/full/10.1056, 2013).

Gene therapy approved by EC

Glybera, a gene therapy treatment for a rare disease in which persons are unable to digest fat, has been approved by the European Commission (EC) for commercial use in Europe. The therapy is the first such to be approved in the West, with the action coming almost 10 years after China approved a recombinant Ad-p53 gene therapy for head and neck squamous cell carcinoma.

The premise of gene therapy is straightforward: Do not simply treat a patient's symptoms if a disease state is caused by genetic mutation. Instead, fix the faulty genetic code that underlies the problem. In the case of Glybera, adeno-associated virus vectors deliver DNA encoding a lipid-processing enzyme to the cells of patients suffering from lipoprotein lipase deficiency (LPLD). LPLD occurs in only one or two persons per million and prevents the body from metabolizing fats in the blood.

UniQure, the Amsterdam-based developer of the therapy, expects the first treatments will be made available to the public in late 2013. The company said it will also seek regulatory approval in the United States and Canada.

New product health, nutrition claims continue to grow

The US Department of Agriculture's Economic Research Service (USDA ERS) found that 43% of new food products in the Unites States in 2010 carried at least one health- and nutrition-related label claim, compared to 25% of new products in 2001 and 35% in 1989.

The reduction in health- and nutrition-related claims from 1989 to 2001 followed enactment of the Nutrition Labeling and Education Act of 1990 (NLEA), which required most food products to carry the Nutrition Facts label and established labeling rules for the use of voluntary nutrient content and health claims. The largest increase in health- and nutrition-related claims, when comparing 2001 to 2010 data, was for "no gluten," followed by "no *trans* fats."

Omega-3 fatty acids and breast cancer

A lifetime of exposure to long-chain omega-3 fatty acids may inhibit the growth of breast cancer tumors—if results in mice generalize to humans—according to research from Canada's University of Guelph (UG; Ontario).

The researchers, led by Mira MacLennan, a former UG graduate student, believe they are the first to provide unequivocal evidence that omega-3s reduce cancer risk.

"It's a significant finding," said David Ma, a professor in UG's Department of Human Health and Nutritional Sciences, and one of the study's authors.

"We show that lifelong exposure to omega-3s has a beneficial role in disease prevention—in this case, breast cancer prevention. What's important is that we have proven that omega-3s are the driving force and not something else."

Advocates have long believed diet may significantly help in preventing cancer, but epidemiological and experimental studies to back up such claims have been lacking, and human studies have been inconsistent, Ma said.

"There are inherent challenges in conducting and measuring diet in such studies, and it has hindered our ability to firmly establish linkages between dietary nutrients and cancer risk," he said. "So we've used modern genetic tools to address a classic nutritional question."

For their study, the researchers created a novel transgenic mouse that both produces omega-3 fatty acids (in the form of docosahexaenoic and eicosapentaenoic acids; DHA+EPA) and develops aggressive mammary tumors. The team compared those animals to mice genetically engineered only to develop the same aggressive mammary tumors.

"This model provides a purely genetic approach to investigate the effects of lifelong omega-3 exposure on breast cancer development," Ma said. "To our knowledge, no such approach has been used previously to investigate the role of omega-3s and breast cancer."

Mice producing DHA+EPA developed only two-thirds as many tumors—and tumors were also 30% smaller—as the control mice did.

"The difference can be solely attributed to the presence of omega-3s in the transgenic mice—that's significant," Ma said. "The fact that a food nutrient can have a significant effect on tumor development and growth is remarkable and has considerable implications in breast cancer prevention."

Funding for the study, which appeared in the *Journal of Nutritional Biochemistry* (*24*:388–395, 2013), came from the Canadian Breast Cancer Research Alliance/Canadian Institutes of Health Research, the Canada Foundation for Innovation, and the Ontario Research Fund. ■

BRIEFS

Monsanto Co. (St. Louis, Missouri, USA) has purchased substantially all of the assets of the Israeli company Rosetta Green Ltd. It has also assumed some of its liabilities. Rosetta Green develops improved plant traits for the agriculture and biofuel industries, using microRNA genes. The company specializes in the identification and use of these genes that function as "main bioswitches" to control key processes in major crops such as corn, wheat, rice, soybean, cotton, canola, and algae. Rosetta Green was formed in 2010 by the spin-off of the agro-biotechnology division of Rosetta Genomics Ltd. (Rehovoth, Israel).

■■■

A senior agricultural official of the People's Republic of China indicated in March that China will continue to import genetically modified (GM) soybeans to satisfy domestic demand (http://tinyurl.com/China-GM-soy). In 2012 the country imported 58.38 million metric tons (MMT) of GM soybeans, mostly from the United States, Brazil, and Argentina. The country produces about 14 MMT of soybeans annually, but the demand exceeds 70 MMT. According to the International Service for the Acquisition of Agri-Biotech Applications, China has the sixth-greatest area in the world devoted to GM planting, 1.6 million hectares. Cotton is grown on the largest portion of that area, followed by papaya, tomato, and sweet pepper.

■■■

Whole Foods Market, a grocery chain in the United States, announced in March that it had set itself a deadline that, by 2018, all products in its US and Canadian stores must be labeled to indicate whether they contain genetically modified organisms (GMO). Currently, Whole Foods sells some 3,300 private-label and branded products that are certified GMO-free. The Grocery Manufacturers Association issued a statement opposing the move, saying, "These labels could mislead consumers into believing that these food products are somehow different or present a special risk or a potential risk." ■

BIOTECHNOLOGY NEWS

Patent rights and biotech seeds

The Supreme Court of the United States (SCOTUS) heard arguments on February 18, 2013, on a case involving patent infringement with respect to genetically engineered (GE) soybeans. Seed producer Monsanto Co., St. Louis, Missouri, sued Vernon Bowman, an Indiana farmer who is receiving legal backing from a number of academics and pro bono attorneys, and two nonprofit organizations, the Center for Food Safety and Save Our Seeds.

A decision on the case is not anticipated until June or July of 2013, but reports of the hearing, discussed below, point to the favorable reception by the justices of the arguments presented by Monsanto.

Background. Monsanto initiated its suit against Bowman in 2007, arguing that he had planted the offspring of its patented GE soybean—which had been bioengineered to resist the herbicide Roundup—in contradiction to the contract that Monsanto makes its seed purchasers sign, agreeing not to replant the original seeds' progeny.

Farmers have a long tradition of saving seeds for replanting. Some have replanted Roundup Ready soybeans anyway and thus have violated the contracts with Monsanto they had to sign in order to purchase and plant these beans. According to the Center for Food Safety (CFS; http://tinyurl.com/CenterFoodSafety), the company has sued farmers 144 times as of January 13, 2013, prevailing in the 11 cases that went to trial. More than 400 farmers in 27 states were involved.

Bowman's approach was different. Instead of replanting Roundup Ready soybeans that he himself had grown, in 1999 he bought commodity beans from the local elevator to plant for a risky late-season crop on which he didn't want to spend much money. He reasoned that many of these commodity beans, which are typically sold as animal feed, would be Roundup Ready, especially in light of the fact that 90% of the soybeans grown in the United States are Roundup Ready (http://tinyurl.com/StLouis-Bowman). And he was correct—they did resist Roundup when he applied that herbicide to his second-crop beans.

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Bowman continued to plant seed harvested from his initial second-crop sowing in later years. He also supplemented his second-crop planting supply with periodic additional purchases of commodity seed from the grain elevator (http://tinyurl.com/SCOTUS-petition). In summary, he did not purchase the second-crop seed from Monsanto and he did not sign a contract with the company for those seeds.

When Monsanto learned of Bowman's activities, which he had made no effort to hide, it sued, arguing that the company "retained rights even though there was no agreement specific to that transaction" (http://tinyurl.com/StLouis-Bowman). Bowman contended that the doctrine of patent exhaustion (the termination of a patent owner's rights to control the use of patented product or a process used for making that product) allowed him to do what he wanted with products he had obtained legally. However, the district court agreed with Monsanto and fined Bowman $84,456. The appellate court concurred in 2011, saying that the seed from the planting was a "newly infringing article." The case then went to the Supreme Court in December 2011 on a request for a writ of certiori (request for re-examination of actions of an inferior appeals court), and the request was granted in October 2012.

SCOTUS hearing. Twenty-three amicus curiae briefs (*amicus curiae*: friend of the court. Thus, a brief presented by someone interested in influencing the outcome of a lawsuit but who is not a party to it) were filed for this hearing, representing organizations such as the National Farmers Union; the Automotive Aftermarket Industry Association; Agilent Technologies; the Biotechnology Industry Organization; Pioneer Hi-Bred International, Inc.; and the American Soybean Association (http://tinyurl.com/ABA-Bowman-Monsanto).

The justices considered the case for only 70 minutes, which *USA Today* said indicated that SCOTUS was strongly in favor of Monsanto's argument (http://tinyurl.com/SCOTUS-70min). *The New York Times* pointed out that Monsanto's lawyer, Seth P. Waxman, a former US solicitor general, "was allowed to talk uninterrupted for long stretches, which is usually a sign of impending victory" (http://tinyurl.com/SCOTUS-Waxman).

Justice Sonia Sotomayor said Bowman could not make his case on the doctrine of patent exhaustion. "The exhaustion doctrine permits you to use the goods that you buy. It never permits you to make another from that item you bought" (http://tinyurl.com/NYT-exhaustion). Justice Stephen Breyer said, "[Y]ou cannot make copies of a patented invention."

Chief Justice John Roberts asked, "Why in the world would anyone spend money to improve the seed if as soon as they sold the first one anybody could grow more" at no extra cost? (*Chicago Tribune*, February 20, 2013).

Justice Elena Kagan pointed out the case could present an opportunity for SCOTUS to clarify its position on conditional sales of patented products (http://tinyurl.com/Kagan-conditional).

According to InsideHealthPolicy (http://tinyurl.com/Kagan-conditional), several justices emphasized that "the court has never heard a patent exhaustion case specifically involving self-replicating technologies."

Implications. The implications of this case go beyond seed production. For example, BSA/The Software Alliance, which

represents companies such as Apple and Microsoft, said in its amicus brief that a decision against Monsanto might "facilitate software piracy on a broad scale" if buyers were free to copy software after a first use (http://tinyurl.com/Monsanto-software).

Entities in the biotechnology field also are watching the outcome of the case. Issues regarding technologies as disparate as stem cells, live vaccines, transgenic animals, DNA used for research or medical treatment, regenerative medicine, and other genetically modified products besides soybeans could be affected by the outcome of this hearing.

On the other hand, the Automotive Aftermarket Industry Association, which represents makers of replacement auto parts, sided with Bowman in its amicus brief, arguing against restricting how patented products are sold and resold.

The journal *Nature* pointed out that if the outcome of this hearing is in favor of Bowman, Monsanto and other companies dependent on self-replicating technologies will be forced to revisit technologies that can short-circuit copycat proliferation of their work (http://tinyurl.com/Nature-Monsanto). One possibility would be to create GE plants that generate sterile seeds (so-called terminator technology). A second might be to engineer the seeds so that they can grow into new plants that do not pass along the benefits of the engineered trait to the next generation of seeds. A third could be to put the gene that has been beneficially altered under the control of a switch that must be activated by a proprietary chemical, one that the seed producers would sell to the farmers each year along with their seeds.

Nature reports that Monsanto is not currently researching these techniques, and that other companies hope they will not have to do so.

Monsanto to sue EFSA?

A controversy started in September 2012 when scientists led by French molecular biologist Gilles-Eric Séralini contended in an article appearing in *Food and Chemical Toxicology* that rats fed Monsanto's NK603 corn, which is tolerant to the herbicide Roundup, had a higher incidence of cancers, larger cancerous tumors, and died earlier than controls (see *Inform 24*:30,47,156, 2013).

The European Commission had approved NK603 for use in feed and food products and in food in 2005.

The European Food Safety Agency (EFSA) reviewed Séralini's paper and concluded it was "of insufficient scientific quality to be considered valid for risk assessment" (http://tinyurl.com/EFSA-review). EFSA reported sending two letters to Séralini in October 2012 requesting further data to use in its evaluation of any safety issues involved with NK603.

Séralini responded by saying he and his co-workers would not release their data until EFSA released the data it had used to authorize the approval of NK603 in Europe. On January 14, 2013, EFSA released almost all of the documents and data that Monsanto had submitted in 2003 for authorization to permit planting its NK603 corn.

Following the release of the EFSA data, Séralini announced on January 15 that he would file "complaints of defamation against claims of 'fraud' and 'falsified data'" regarding the September data that appeared in French newspapers.

In the latest installment of this saga, the Parisian newspaper *Le Monde* reported on March 8, 2013, that Monsanto was threatening to sue EFSA for publishing the data used to render an opinion favoring the marketing of NK603 (http://tinyurl.com/LeMonde-GMO). Corinne Lepage, member of the European Parliament and former Minister of the Environment for France, denounced Monsanto's effort to "maintain *omerta* [code of silence] on the raw data of GMOs."

She added, "This transparency of the raw data is not only legitimate but also perfectly legal, since [a European law] precludes confidentiality studies relating to the impact on health and the environment of GMOs." ■

Biofuels News (cont. from page 294)

a premium for algae-derived fuel (http://tinyurl.com/algae-Propel-Solazyme). The fuel was sold at the same price as conventional diesel.

Genetic diversity of jatropha

SGB Biofuels has confirmed to its satisfaction that *Jatropha curcas* has a genetic diversity comparable to corn and other domesticated crops. This indicates there is plenty of room to achieve significant yield and performance gains through molecular breeding for this oil-producing plant.

Eric Mathur, chief technologist at SGB (San Diego, California, USA), said in a company statement, "Based on these results, the performance of our first-generation hybrids merely scratches the surface of the underlying genetic potential of jatropha, not only in terms of continued yield improvements but also through reduction of input costs and improvement of the harvest index."

SGB's molecular and genetic technologies have advanced to the point where millions of genetic markers can be analyzed from a large number of plants within a short period of time. The company is now starting a large-scale jatropha re-sequencing program designed to associate agronomic traits and plant attributes with genome-wide markers. The resulting dense genetic maps will accelerate the rate of improvement of SGB's hybrid cultivars.

New approach to growing algae

In the classical method for growing algae to produce oil, the accepted wisdom is to deprive algae of key nutrients such as nitrogen to boost their oil content. However, recent research has found that it may be better to tolerate lower oil content in algae in order to boost growth and overall productivity.

According to Stephen Wilkinson and his team in the Department of Chemical and Biological Engineering at the University of Sheffield (United Kingdom), "You get more oil production overall if you give the algae all nutrients they need to grow fast rather than trying to increase the oil in each cell by limiting the availability of nitrogen."

The scientists grew the alga *Dunaliella salina* at different cell densities over a range of temperatures to determine the rate of growth and lipid production. Some samples were deprived of nitrogen, others were not. During the four-week study, the overall yield from the nitrogen-starved crops was lower than many of the crops that had been allowed to grow naturally.

The researchers also found that increasing the cell density led to greater productivity. Cell density was increased by using centrifugation to create more crowded algal cultures, and unexpectedly Wilkinson and co-workers found that these samples could still grow very well.

The study appeared in the *Journal of Chemical Technology and Biotechnology* (DOI:10.1002/jctb.4027, 2013).

NASA's flying laboratory tests biodiesel fuel emissions

Using its DC-8 flying laboratory, researchers with the US National Aeronautics and Space Administration (NASA) tested and compared the emissions and aircraft-generated contrails created from jet fuels in California during early March 2013. The flights evaluated conventional kerosene-based JP-8 jet fuel, comparing it with a 50:50 blend of JP-8 and an alternative fuel of hydroprocessed esters and fatty acids derived from camelina oil.

The Alternative Fuel Effects on Contrails and Cruise Emissions (ACCESS) research involved flying the DC-8 as high as 40,000 feet (12,000 m) while another NASA aircraft trailed behind at distances ranging from 300 feet (90 m) to 10 miles (16 km). The trailing aircraft characterized the soot and gases streaming from the DC-8, monitored exhaust plumes as they mixed with the air, and investigated the role emissions play in contrail formation.

ACCESS follows a pair of studies conducted in 2009 and 2011 in which ground-based instruments measured the DC-8's emissions from alternative fuels while it sat parked on a flight ramp. A second phase of ACCESS is already planned for 2014 that will take into account details from the March 2013 tests.

Shipping biodiesel blends by pipeline

Colonial Pipeline, an interstate common carrier of petroleum products headquartered in Alpharetta, Georgia, USA, daily delivers an average of 100 million gallons of gasoline, kerosene, home heating oil, diesel fuel, and national defense fuels to shippers in 13 states and the District of Columbia.

In mid-March 2013 the company announced its intention to begin shipping biodiesel blends through its pipeline to central and southern Georgia by the end of the month, calling it a first for the company. The blend to be shipped will have a composition of 5% or less of biodiesel, suitable for trucking, farming, and other diesel uses. According to Platts Oilgram News, the biodiesel will be stored in tankage owned by Trans-Montaigne Partners and injected into diesel as it flows through Colonial's pipeline (http://tinyurl.com/Colonial-Platts).

Until now, Colonial Pipeline has shipped renewable diesel, but not biodiesel, for fear that it would "trail back" into any jet fuel back being transported in the pipeline, causing problems in airplane engine performance. The line in which the biodiesel will be shipped does not have any distribution to airlines. For example, it does not go anywhere near the international airport at Atlanta, Georgia. ■

BRIEFS

The UK's Croda International Plc is the world's second-largest cosmetic-ingredient maker and is closing the gap on BASF, according to the Bloomberg news service. Croda reportedly is gaining market share by "snagging smaller customers that fall under the radar," CEO Steve Foots was quoted as saying. "Croda's consumer-care division reported an 8.3% increase in earnings last year on a 2.6% advance in sales. By contrast, BASF's care-chemicals division, which encompasses Cognis, reported a 4.2% decline in revenue," Bloomberg noted.

■■■

Swiss specialty chemical producer Clariant has opened a new 2-acrylamido-2-methylpropane sulfonic acid (AMPS) polymer plant for production of specialty polysulfonates at its facility in Tarragona, Spain. The plant will produce two lines of AMPS polymers for the growing cosmetics and oil and gas drilling aids markets. The €16 million ($21 million) project incorporates modern process technology to save energy, according to Clariant.

■■■

Shell Chemical is thinking of adding a fourth linear α-olefin unit at its Geismar, Louisiana, USA, petrochemical facility, according to ICIS news. The addition "is intended to help meet growing global demand for products such as polyethylene co-monomers, lubricants and lubricant additives, surfactants, and offshore drilling fluids," ICIS said.

■■■

Global use of dry shampoo has increased over the past five years, according to the Mintel Beauty and Personal Care Global New Products Database. Dry shampoo, generally a powder of talc or silica placed in the hair to remove natural oils without water, accounted for only 1% of global new shampoo product introductions in 2008. That share grew to 3% by 2012, with 2013 on track to surpass 2012 levels. However, in what is good news for surfactant manufacturers and shampoo formulators, consumer uptake remains relatively low. Only 16% of US adults reported some use of a dry shampoo in 2012, and usage levels in major European markets were relatively similar to US usage, reaching a high of 23% of adult women in the UK. ■

SURFACTANTS, DETERGENTS, & PERSONAL CARE NEWS

Emu oil target of counterfeiters

Cosmetic and personal care formulators have used emu oil as a transdermal carrier of bioactive ingredients and as a moisturizer with anti-inflammatory properties. But with oil from the flightless bird selling at wholesale for between $90 and $130 per kilogram, emu oil adulteration remains a problem for both the emu industry and product formulators.

The American Emu Association (AEA; Ottawa, Illinois), a trade association founded in 1989, has been working for more than a decade to counter the counterfeiters. In 2000, AEA set in place several programs to fight adulteration. First, AEA released Trade Rule 102 specifying testing procedures, based on AOCS methods, for grading emu oil (see http://tinyurl.com/emu-rule102). Next, the group developed Trade Rule 103 (see http://tinyurl.com/emu-rule103), which classifies three grades of emu oil: Grade A (fully refined oil), Grade B (once-refined oil), and Grade C (crude oil).

The group also introduced a certification program in 2000. To be certified as being fully refined, oil must be processed in an AEA-certified emu oil refinery—where each batch is registered—and be bottled by an AEA-certified bottler. Further, a sample from each independently tested, fully refined Grade A batch must be held in storage by the refiner so that it can be pulled for testing if there is any question about product purity.

In 2012, AEA conducted a small, unpublished study to test for adulteration in noncertified emu oil. The group purchased oil from 11 different companies including two samples from Australian vendors. After analyzing the fatty acid profiles of all 11 samples, six were deemed to be adulterated, based on their fatty acid profiles.

Paul Binford, an AOCS member and former head of research at AEA, runs one of the largest emu processing plants in the United States, LB Processors LLC in Chapmansboro, Tennessee, USA. The LB plant produces roughly 7,000 gallons (28,000 liters) per year.

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"I bought a five-gallon bucket of what was supposed to be emu oil off the Internet," he said. "It looked like soybean oil, which is what it turned out to be." Ersatz emu oil often actually is whichever vegetable oil is the cheapest at the moment, he said. "The best way to be sure you are purchasing emu oil," he stressed, "is to buy AEA-certified oil."

Australia, another large producer of emu oil, does not have a similar problem with adulteration, according to Chris Gregory, president of the Emu Industry Federation of Australia.

"To my knowledge, there have been no instances of counterfeit oil placed on the market and I have not heard of any complaints," Gregory said.

Argan oil benefits consumers, producers, environment

In Morocco, two resources are aiding economic and social change: argan oil and the Amazigh women who produce it.

Argan oil is produced from argan tree seeds and contains roughly 80% oleic (18:1n-9) and linoleic (18:2n-6) fatty acids. It is known as an effective moisturizer and emollient for skin and hair, which has driven its global use in cosmetics and hair treatments. It is also one of the main ingredients of the Moroccan food *amlou*, a spread or dip of argan oil, ground almonds, and honey.

The Amazigh people—also known as "Berbers"—are the indigenous ethnic group of North Africa west of the Nile River. In the past, Amazigh women rarely left the home to work, but now 170 cooperatives that produce argan oil employ about 5,000 women, according to Cable Network News (CNN).

"When we started in 1996 we had [. . .] only 16 women who believed in our project. Most of them were divorced or without a husband," said Zoubida Charrouf, a chemistry professor at Mohamed V University in Rabat, Morocco, who helped found one of the first cooperatives in the region in conjunction with the International Development Research Center (IDRC; Ottawa, Ontario, Canada). She wanted not only to protect the argan tree (*Argania spinos*a L.), which was in danger from deforestation, but also to uplift the Amazigh women. Through the co-ops, the women have been able to bond and share stories, collect wages, and in general exert more power over their lives.

"These women are not the same as before," Charrouf said in an IDRC news release. "Together, they share their problems, their laughter. And because they are together, the project can offer training in literacy, marketing, quality control, and other subjects."

Argan oil production represents one of the few opportunities for women with limited technical skills and training to earn income outside the house. In traditional production, the argan fruit is left out to dry for several days before the pulp is peeled away to reveal anywhere from one to three nuts. These nuts must be cracked to extract the oil, but all attempts to mechanize the process have been unsuccessful so far because the number of kernels in the nut varies.

The Amazigh women first use jagged stones to crack the shells and then place the kernels between two rocks, grinding them into a brown, peanut butter-like paste. They knead the paste to extract the oil, leaving a solid block that goes off to a mechanical press for further extraction. It takes about 20 hours to produce just one liter, according to CNN.

The argan tree grows in arid and semi-arid landscapes, and part of Charrouf's project includes replanting and protecting the trees, whose deep root systems prevent desertification. Up to 60,000 new argan trees are planted each year, she says.

The United Nations Educational, Scientific, and Cultural Organization has declared the 26,000 square-kilometer argan-growing region as a "biosphere reserve" under the Man and the Biosphere program. This designates the growing region as a protected area and recognizes it as having important economic and environmental value (http://tinyurl.com/UNESCO-reserve).

But there are some questions regarding the net effect of argan oil production. Research published in *Proceedings of the National Academy of Sciences* (doi:10.1073/pnas.1106382108, 2011) claims that although the argan oil industry has led to the improvement of educational outcomes and financial stability for women in the short term, it is having long-term negative effects on the argan tree forests.

In the article, a team led by Travis J. Lybbert of the University of California, Davis (USA) agrees that the argan industry has increased the income of many rural households. But this, in turn, has increased the size of goat herds, which graze on the leaves of the low-growing argan tree, negatively affecting future argan fruit harvests. Inefficient harvesting methods and increased use of the tree for fuel also cause damage.

Further, although educational opportunities have also improved for Moroccans, particularly women, this may not be beneficial in the end, Lybbert and co-workers argue. Education may increase, but different opportunities must also increase, according to the team.

At least one Amazigh woman, however, believes that demand for argan oil is improving the lives of the people who produce it.

"We've started relying on ourselves," Ichou Aisha, a co-op worker, told CNN. "Now, we can provide for our children on our own." ■

[FAST FACT]

A little Calorease with that pie a la mode?

The six-glucose fiber molecule α-cyclodextrin, also known as fat-binding complex, FBC_x, is the active ingredient in a new dietary supplement that the Indonesian/Australian supplement and pharmaceutical company SOHO Flordis International plans to market in North America under the Calorease brand name. According to Nutraingredients-USA.com, most fiber molecules combine with fat at a 1:1 ratio, but a single FBC_x molecule can form a stable bound unit with as many as nine fat molecules—*and* the ingredient binds preferentially to saturated and *trans* fats before being eliminated from the body like any other undigestible plant fiber. More information is available at http://tinyurl.com/FatAbsorption.

PEOPLE NEWS

Willits retires

James (Jim) E. Willits, chairman of the board for Desmet Ballestra North America, retired on March 31, 2013, after serving nine years in the Oil & Fats Division of the Desmet Ballestra group.


Willits

Willits entered the agribusiness field 40 years ago as a chemist and laboratory supervisor for Grain Processing Corp. He moved on to the oils and fats industry in 1978, when he accepted a position with Central Soya. While there, he worked in multiple plant sites, gaining experience and responsibility as a quality control superintendent, refinery superintendent, and plant manager. In 1989 he accepted the position of vice president of production for Karlshamns' three US production facilities. In 2001, Willits moved to sales, first working for Alfa Laval, and then accepting the position of vice president of sales for Desmet Ballestra North America in 2004 and in 2011 was promoted to president and chief executive officer. This progression of responsibilities gave him the opportunity to work on projects in many parts of the world.

During the course of his career, Willits has been committed to advancing the field of oils and fats. He has been a returning instructor at the Texas A&M University *Short Course Series on Vegetable Oil Processing*, and has presented at international conferences.

Willits has been an active participant in AOCS since he joined in 1979. He has served for many years as an officer in the Processing Division (chair, vice chair, secretary, treasurer, member-at-large) and has also been a member of various committees determining winners of AOCS awards. He has contributed regularly to AOCS-sponsored short courses, chaired sessions at annual meetings, and given presentations as well.

In retirement, Willits plans to move to his farm in Ohio and spend time with his family. He has also established a consulting firm called Willits Oils & Fats Consulting LLC, and he may be contacted at jwillits65@gmail.com.

Eurofins appoints new president

AOCS member **Mary Kay Krogull** was hired as president of the US Food Division of Eurofins Scientific in March 2013. She will lead the strategic development and scientific oversight of the North American Eurofins Food Testing operations.


Krogull

Krogull comes to Eurofins with over 30 years of experience in academia and the food science industry. She has an M.S. in food chemistry from the University of Wisconsin, and before her appointment at Eurofins was global vice president at Covance. Much of her career has been with testing services.

Eurofins provides services in the area of food testing that include comprehensive chemical and microbiological testing with food safety auditing.

Morris becomes president of oilseed processing, ADM


Morris

At the end of February, Archer Daniels Midland Company (Decatur, Illinois, USA), announced it had promoted **Gregory Morris** to the newly created role of president, North American Oilseed Processing. He previously served as vice president of the business.

In this new position Morris will continue to oversee all of the company's North American Oilseeds processing business including more than two dozen crushing plants where the company turns soybeans, cottonseeds, and various softseeds, such as canola and sunflower, into meal and oil. He will also oversee ADM's Specialty Products business, which includes the company's Protein Specialties, Lecithin, and Natural Health and Nutrition divisions.

Morris joined ADM in 1995 and has held several merchandising and management positions since that time.

Nucelis appoints new president/CEO

In February **Sean P. O'Connor** was appointed president and CEO of Nucelis Corp. (New York City, New York, USA). He comes to Nucelis from Chemtura Corp., as well as British Petroleum.

Nucelis is a specialty chemicals company with a patented technology for modifying cell structure and function. The technology has potentially significant value in industries turning to sustainable and renewable products and chemistry, such as the cosmetics industry, products for the food and fragrance industries, and specialty edible oils with particular health benefits.

Their technology, the Rapid Trait Development system (RTDS™), relies on the cell's own repair mechanism to implement changes, rather than inserting foreign materials into a cell's DNA sequence. RTDS has been certified as nontransgenic by the US Department of Agriculture. This categorization may give

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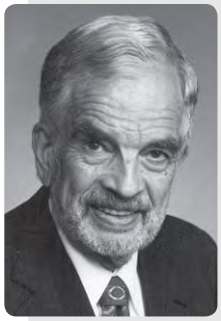
IN MEMORIAM

EDWARD CHARLES LEONARD



Charles Leonard, a long-time member of AOCS and well-known civic volunteer in Memphis, Tennessee, USA, died on January 31, 2013, at the age of 85. Evelyn, his wife of 60 years, survives him, as well as many friends.

Leonard received a Ph.D. in organic chemistry from the University of North Carolina in 1951 and a Master's in Business Administration from the University of Chicago.

His first position out of graduate school was as a chemist with the Union Carbide Corp. He rose to group leader before leaving to join Borden Chemical Co. in 1964. He moved to a managerial position with National Dairy Products Corp. (Glenview, Illinois) in 1967, the same year he joined AOCS.

Leonard became technical director of Humko-Sheffield, a division of Kraftco, in the early 1970s, and stayed with the company through its various name changes until his retirement from the Humko Chemical Division of Witco Corp. in 1993 as general manager/group vice president of the oleochemical group.

In his retirement he established Hume Co. (Botanoceuticals, Inc.), specializing in medical chemicals and botanical products. Most of the patents that were awarded to Leonard came from his work with Botanoceuticals.

Leonard was an active member of the Surfactants and Detergents Division of AOCS. He chaired AOCS short courses on fatty acids in 1979 and 1984, chaired sessions at annual meetings, and presented papers at meetings as well. His analyses of the economics of oleochemicals were especially well received. He served on the editorial advisory boards for the *Journal of the American Oil Chemists' Society, Journal of Surfactants and Detergents*, and *Inform*.

He also made significant contributions to AOCS-sponsored World Conferences. Leonard chaired the October 1990 World Conference on Oleochemicals, held in Kuala Lumpur, Malaysia; the February 1994 World Conference and Exhibition on Lauric Oils: Sources, Processing, and Applications, held in Manila, the Philippines; and the February 1998 World Conference and Exhibition on Palm & Coconut Oils for the 21st Century, held in Bali, Indonesia. He also shepherded the proceedings of these conferences into print.

Besides AOCS, Leonard was active in the Soap and Detergent Association (SDA), and received its lifetime achievement award, presented by the Glycerine and Oleochemical Division, at SDA's 1994 annual convention.

In 1993, Leonard received the AOCS Award of Merit, given for meritorious committee work and marked leadership in administrative activities.

CARL FREDERICK BROWN

Carl F. Brown, of Westfield, New Jersey, USA, died on December 1, 2012, at the age of 94. Brown graduated from Lehigh University in Bethlehem, Pennsylvania (USA), earning a B.S. in chemical engineering and a B.A. in history and government. He worked as a chemical engineer for Best Foods in Union, New Jersey, for 41 years before retiring.

He is survived by his wife of 63 years, Elizabeth, and cousins and friends.

Brown joined AOCS in 1949, and received emeritus status in 1983. ■

Nucelis a protected competitive position in areas such as Europe, where "genetically modified" is not well accepted.

New director of engineering

Distaso

Pump Solutions Group (PSG), a business unit within Dover Corporation and manufacturer of positive displacement pumps and related technologies, named **Chris Distaso** in February as director of engineering for PSG's Grand Terrace facility, which is located in Grand Terrace, California, USA. Distaso will be responsible for the overall supervision and management of the research and development function as well. Distaso will report to **Denny Buskirk**, general manager of PSG's Grand Terrace facility.

CLASSIFIED

More Patents can be found in the supplement to the digital edition of Inform. *Log in to read the May 2013 issue at www.aocs.org/login.*

PATENTS

Method of preparing a microcapsule containing unsaturated fatty acids, the microcapsule prepared by the method, and articles containing the microcapsule

Sin, H.-S., *et al.*, Chebigen Inc., US8282961, October 9, 2012

The present invention provides a preparing method of microcapsule containing unsaturated fatty acid comprising the steps of (i) preparing a first coating material by mixing and gelatinating one or more gums selected from the group consisting of xanthan gum, guar gum, and locusbean gum, with poly glycerin esters of fatty acid in sterilized water, adding unsaturated fatty acid to the solution, and homogenizing the solution; (ii) preparing a second coating material by mixing and gelatinating starch or modified starch, gelatin or casein, and poly glycerin esters of fatty acid in sterilized water, adding the first coating material prepared in (i) to the solution, and homogenizing the solution; and (iii) spraying the second coating material prepared in (ii) in cold sterilized water; a microcapsule prepared by the method; and articles containing the microcapsule. The present microcapsule has effects of preventing the oxidation of unsaturated fatty acid and inhibiting offensive smell.

Chocolate composition

Bruse, F., *et al.*, Cargill, Inc., US8293314, October 23, 2012

A chocolate composition having a fat phase, characterized in that said fat phase: has a slip melting point of at least 27.5°C, preferably of between 30 and 37°C; and comprises both modified and unmodified fats, wherein said modified fats comprise an interesterified fat consisting of interesterified cocoa butter.

Methods and compositions for improving air entrainment in cementitious mixtures

White, C.M., *et al.*, Cognis IP Management GmbH, US8287639, October 16, 2012

A method for improving air entrainment comprising the steps of: providing a compound selected from an amphoteric, an alkyl polyglycoside, an ester, a triglyceride, a triglyceride derivative, a fatty alcohol, an alkoxylated fatty alcohol, an alkoxylated polyhydric fatty alcohol, and mixtures thereof; dispersing the compound on a finely particulate carrier to form a treated carrier; and adding the treated carrier to a cementitious mixture is provided. A composition for improving air entrainment, including a compound selected from an amphoteric, an alkyl polyglycoside, an ester, a triglyceride, a triglyceride derivative, a fatty alcohol, an alkoxylated fatty alcohol, an alkoxylated polyhydric fatty alcohol, and mixtures thereof, wherein the compound is dispersed on an organic particulate carrier and added to a cementitious mixture, is also provided. The composition may be incorporated into a concrete structure.

Modified inorganic particles for deinking

Rosencrance, S., *et al.*, Kemira Chemicals, Inc., US8287690, October 16, 2012

The present invention provides methods for deinking printed waster paper—particularly methods that use a deinking composition that includes a hydrophobically-modified inorganic particle (MIP) to improve ink collection efficiency under traditional alkaline, reduced alkali, and true neutral deinking conditions. Deinking compositions are provided that include a hydrophobically-MIP substrate, a nonionic surfactant, and a fatty acid, or mixtures thereof. The improved ink collection of the present invention can result in deinked pulp of high quality and/or yields that has excellent brightness and effective residual ink concentrations (ERIC) values.

Bitterness-masking particulate jelly beverage

Fukui, A., Ryukakusan Co., Ltd., US8287897, October 16, 2012

A particulate jelly beverage which facilitates the intake of a bitter drug and/or supplement. The beverage comprises 0.1 to 15.0% bitterness-masking ingredient comprising a vegetable fat or animal fat, 5 to 20% bitterness-masking aid comprising a sugar alcohol, 0.1 to 5.0 % gellant such as an agar or carrageenan, and water as the remainder. It may optionally contain an ingredient for reducing water repellency, such as a sucrose/fatty acid ester, glycerol/fatty acid ester, or propylene glycol, in an amount of 0.01 to 1.5%.

Dry analytical element for lipase measurement

Nakamura, K., and S. Kageyama, Fujifilm Corp., US8288117, October 16, 2012

It is an object of the present invention to provide: a dry analytical element for analyzing pancreatic lipase wherein the triglyceride is not transcribed on the support to contaminate a transportation slip or other analytical elements and wherein an additive solution of the triglyceride neither reaggregates nor precipitates, so that the dry analytical element is stable and is compatible with production. The present invention provides a dry analytical element for measuring pancreatic lipase contained in body fluid, which comprises triglyceride of long-chain alkyl fatty acid having 12 to 22 carbon atoms, monoglyceride lipase, and a glycerin measurement reagent, and which comprises

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AOCS MEETING WATCH

July 16–17, 2013. AOCS Technical Services Workshop: Laboratory Methods, Des Moines Ankeny, Iowa, USA. www.aocs.org/labworkshop

August 8–10, 2013. International Conference on Emerging Trends in Oleochemicals & Lipids Expo 2013, Hyderabad, India. www.aocs.org/oleo2013

August 20–23, 2013. XV Latin American Congress and Exhibition on Fats and Oils, Sheraton Santiago Hotel and Convention Center, Santiago, Chile. http://lacongress.aocs.org

November 6–8, 2013. Australasian Section AOCS Biennial Meeting and Workshops, NOAH's on the Beach, Newcastle, New South Wales, Australia. www.aocs.org/australasian

May 4–7, 2014. 105th AOCS Annual Meeting & Expo, Henry B. Gonzalez Convention Center, San Antonio, Texas, USA. http://aocs.org/meetings

October 6–9, 2014. World Conference on Fabric and Home Care: Montreux 2014, Montreux Music & Convention Centre, Montreux, Switzerland. http://Montreux.aocs.org

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

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

PUBLICATIONS

a water-impermeable support and at least one spreading or reagent layer, wherein a hydrophilic polymer at a weight ratio of 1.8:1 or greater with respect to the triglyceride is contained.

Process for making a detergent composition comprising a hydrophilic silica and a copolymer containing a carboxylic acid monomer and a sulfonic acid monomer

Somerville Roberts, N.P., Procter & Gamble Co., US8288333, October 16, 2012

A process for making a detergent composition, the detergent composition comprising: (a) from about 0.1% to about 50% by weight of the composition of a polymer in particulate form comprising: (i) a carboxylic acid monomer; (ii) more than about 5% by weight of the polymer of a sulfonic acid monomer; and (iii) optionally a non-ionic monomer; and (b) from about 0.01% to about 10% by weight of the composition of a hydrophilic silica; the process comprising the steps of: (a) pre-mixing the polymer with the hydrophilic silica to obtain a polymer/silica premix; and (b) mixing polymer/silica premix with any additional detergent components.

Isolated peptides having phospholipase inhibitory activity

de Naria, L., *et al.*, Novozymes A/S, US8288510, October 16, 2012

The invention provides for isolated peptides having phospholipase inhibitory activity, polypeptides comprising phospholipase inhibitory activity and lipases capable of being inhibited by the isolated peptides and/or polypeptides comprising phospholipase inhibitory activity. The invention also relates to nucleic acid constructs, recombinant expression vectors, and recombinant host cells comprising the polynucleotides as well as methods for producing and using the peptides and the polypeptides having lipase inhibitory activity.

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.

EXTRACTS & DISTILLATES

The effects of microwave heating on edible oils and lipid-containing food

Inchingolo, R., *et al., Lipid Technol. 25*:59–61, 2013.

Lipids and lipid-containing foods are particularly sensitive to microwave heating as the specific heat of lipids is low and thus they are quickly warmed up. Microwave heating mainly promotes lipid oxidation, but it can also cause lipolysis and polymerization. This cooking method can differently impact lipid oxidation depending on the treatment conditions used (power, temperature, and time), as well as on food composition. This review provides a picture of the main degradation effects of microwave heating on vegetable oils and lipid-containing foods with emphasis on both fatty acids and cholesterol oxidation.

Using stable isotopes to trace diet-induced shifts in pathways of lipid metabolism

Pu, S., *et al., Lipid Technol. 25*:63–66, 2013.

Advances in the use of stable isotopes in tracer methodology have clearly improved our capacity to investigate *in vivo* lipid metabolism in humans. The introduction of the isotope ratio mass spectrometry (IRMS) provides higher precision of measurement of isotopic enrichment to track shifts in diet on the flux of tracers through metabolic pathways. Novel applications using stable isotopes including ^{2}H, ^{13}C, and ^{18}O have allowed important discoveries to be made in areas of fat and cholesterol metabolism in ways that have assisted in understanding diet-disease relationships.

Determination of total plasma hydroperoxides using a diphenyl-1-pyrenylphosphine fluorescent probe

Santas, J., *et al., Anal. Biochem. 434*:172–177, 2013.

Plasma hydroperoxides (HP) are widely accepted to be good indicators of oxidative stress. By means of the method proposed here, which uses diphenyl-1-pyrenylphosphine (DPPP) as a fluorescent probe, all types of plasma HP were determined. The limits of detection and quantification of the method were 0.08 and 0.25 nmol of cumene hydroperoxide (CHP) equivalents in 40 μL of plasma, respectively. The method is satisfactory in terms of precision (5.2% for 14.5 μM CHP eq., $n = 8$), and the recoveries were 91% and 92% after standard additions of 26 and 52 μM CHP, respectively. The selectivity of the proposed method is higher than 96%. Moreover, optimization of the reaction conditions and the addition of ethylenediaminetetraacetic acid (EDTA) disodium salt and 2,6-di-*tert*-butyl-4-methylphenol (BHT) prevented the formation of HP artifacts during the analysis. Therefore, the proposed method is useful for simple and quantitative determination of total plasma HP.

Selective concentration of EPA and DHA using *Thermomyces lanuginosus* lipase is due to fatty acid selectivity and not regioselectivity

Akanbi, T.O., *et al., Food Chem. 138*:615–620, 2013.

The selectivity of anchovy oil hydrolysis was optimized for *Thermomyces lanuginosus* lipase, so that docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were concentrated and partially separated from each other. Enzyme concentration and pH control were important factors for effective hydrolysis. Monitoring percent hydrolysis using capillary chromatography with flame ionization detector (Iatroscan) and fatty acid selectivity using gas chromatography (GC) indicated that during hydrolysis DHA primarily remained on the glycerol backbone, while EPA was progressively removed. ^{13}C Nuclear magnetic resonance (NMR) data showed that selectivity of hydrolysis was primarily due to fatty acid selectivity and not regioselectivity, with hydrolysis from both *sn*-1,3 and *sn*-2 sites being equally favored.

Assessment of archaeol as a molecular proxy for methane production in cattle

McCartney, C.A., *et al., J. Dairy Sci. 96*:1211–1217, 2013.

The objective of this study was to assess archaeol, a membrane lipid present in methanogenic Archaea, in cattle feces as a molecular proxy for methanogenesis in the rumen. Feces from 16 heifers either in early lactation [71 d in milk (DIM)] or mid lactation (120 DIM), consuming a diet consisting of 30:70 grass silage/concentrates [dry matter (DM) basis], were analyzed for archaeol. To prepare the feces for analysis, total lipids were obtained by Bligh-Dyer extraction, polar head groups were removed by acid methanolysis, an alcohol fraction was obtained by column chromatography, and finally, the alcohol fraction was trimethylsilylated before analysis by gas chromatography-mass spectrometry. Archaeol was quantified by comparison to an internal standard. A highly significant positive relationship was found between fecal archaeol

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concentration (mg/kg of DM) and methane output (g/kg of DM intake). A highly significant effect of stage of lactation on this relationship was observed. The significant relationship was surprising, given the lack of agreement between methane and total methanogens in previous studies using molecular biology techniques. Variation in the relationship between fecal archaeol concentrations and methane output could be attributed to differences in the methane-producing capability per cell and the selective retention of methanogens in the rumen. The effect of stage of lactation may have been due to differences in DM intake, affecting rumen passage rates and, thus, methanogen populations and activities.

Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses

Schwingshackl, L., and G. Hoffmann, *Nutrients 4*:1989–2007, 2012.

No dietary recommendations for monounsaturated fatty acids (MUFA) are given by the National Institute of Medicine, the United States Department of Agriculture, European Food and Safety Authority, and the American Diabetes Association. In contrast, the Academy of Nutrition and Dietetics and the Canadian Dietetic Association both promote <25% MUFA of daily total energy consumption, while the American Heart Association sets a limit of 20% MUFA in their respective guidelines. The present review summarizes systematic reviews and meta-analyses of randomized controlled trials and cohort studies investigating the effects of MUFA on cardiovascular and diabetic risk factors, cardiovascular events, and cardiovascular death. Electronic database Medline was searched for systematic reviews and meta-analyses using. monounsaturated fatty acids, monounsaturated fat, and dietary fat as search terms with no restriction to calendar date or language. Reference lists and clinical guidelines were searched as well. Sixteen relevant papers were identified. Several studies indicated an increase of HDL (high density lipoprotein)-cholesterol and a corresponding decrease in triacylglycerols following a MUFA-rich diet. The effects on total and LDL (low density lipoprotein)-cholesterol appeared not consistent, but no detrimental effects on blood lipids were observed. Values for systolic and diastolic blood pressure were found to be reduced both during short- and long-term protocols using high amounts of MUFA as compared to low-MUFA diets. In type 2 diabetic subjects, MUFA exerted a hypoglycemic effect and reduced glycosylated hemoglobin in the long term. Data from meta-analyses exploring evidence from long-term prospective cohort studies provide ambiguous results with respect to the effects of MUFA on risk of coronary heart disease (CHD). One meta-analysis reported an increase in CHD events; however, most meta-analyses observed a lesser number of cases in participants subjected to a high-MUFA protocol. Although no detrimental side effects of MUFA-rich diets were reported in the literature, there still is no unanimous rationale for MUFA recommendations in a therapeutic regimen. Additional long-term intervention studies are required to characterize efficacy and effectiveness of recommending a MUFA-rich diet among general and clinical populations.

Fast chemical fingerprinting analysis for biodiesel/diesel blends using commercial solid phase extraction (SPE) cartridge and gas chromatography-mass spectrometry (GC-MS)

Ruan, X.C., *et al.*, *Anal. Methods 5*:1205–1213, 2013.

Commercially available solid phase extraction (SPE) cartridges were combined with GC-MS for fast cleanup and fractionation of biodiesel/diesel samples in oil fingerprinting analysis. Several commercially available SPE cartridges [e.g., silica gel/cyanopropyl (SiO_2/C_3-CN) and extractable petroleum hydrocarbons (EPH) fractionation cartridges] were employed for the fractionation of biodiesel/diesel blends into aliphatic, aromatic hydrocarbons, and fatty acid methyl esters (FAME). The SiO_2/C_3-CN (1 g/0.5 g) SPE cartridge could not separate biodiesel from petroleum hydrocarbons effectively, however, the 5 g EPH SPE cartridge successfully separated blended samples into aliphatic, aromatic, and FAME fractions by eluting with 15 mL of hexane, 15 mL of dichloromethylene (DCM)/hexane (1:1, vol/vol), and 18 mL of DCM, respectively. No cross-elution was observed among aliphatic, aromatic and FAME fractions when the loading mass of blends was less than 8 mg. The relative standard deviations (RSD) for five replicate SPE-GC-MS analyses of 5 mg of diesel blended with 20% biodiesel (vol/vol) and soybean oil ranged from 3.4% to 33.2%, 4.3% to 18.7%, and 5.4% to 29.2% for selected alkane, polycyclic aromatic hydrocarbons (PAH), and FAME, respectively. The final recovery rates of surrogates for aliphatic, aromatic, and FAME fractions ranged from 82.5% to 91.1%. This developed method was successfully used for the fingerprinting analysis of three simulated blend samples. The concentration profiles of target compounds and the blended level are both comparable to those obtained by the conventional silica gel column-GC-MS method.

Membrane domains and the "lipid raft" concept

Sonnino, S., and A. Prinetti, *Curr. Med. Chem. 20*:4–21, 2013.

The bulk structure of biological membranes consists of a bilayer of amphipathic lipids. According to the fluid mosaic model proposed by Singer and Nicholson, the glycerophospholipid bilayer is a two-dimensional fluid construct that allows the lateral movement of membrane components. Different types of lateral interactions among membrane components can take place, giving rise to multiple levels of lateral order that lead to highly organized structures. Early observations suggested that some of the lipid components of biological membranes may play active

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- Rapid assessment of quality parameters in cocoa butter using ATR-MIR spectroscopy and multivariate analysis, Maurer, N.E., and L. Rodriguez-Saona
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- Optimization of enzymatic synthesis of tricaprylin in ionic liquids by response surface methodology, Pan, Q., L. Yang, and X. Meng
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- The formation of a 12-hydroxystearic acid/vegetable oil organogel under shear and thermal fields, Co, E., and A.G. Marangoni
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- Reduction of free fatty acids in acidic nonedible oils by modified K10 clay, Pires, J., B. Brasil, and M.E.M. Araújo
- Esterification of fatty acids in greases to fatty acid methyl esters with highly active diphenylamine salts, Ngo, H.L., H. Vanselous, G.D. Strahan, and M. Haas
- Comparison of lipid extraction from microalgae and soybeans with aqueous isopropanol, Yao, L., S.-L. Lee, T. Wang, and J.A. Gerde
- Application of ultrasound for oil separation and recovery of palm oil, Juliano, P., P. Swiergon, R. Mawson, K. Knoerzer, and M.A. Augustin
- Quality of rapeseed oil produced by conditioning seeds at modest temperatures, Kraljić, K., D. Škevin, M. Pospišil, M. Obranović, S. Neđeral, and T. Bosolt
- Acrolein production from crude glycerol in sub- and super-critical water, Cheng, L., L. Liu, and X.P. Ye

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- n-3 Polyunsaturated fatty acids: relationship to inflammation in healthy adults and adults exhibiting features of metabolic syndrome, Robinson, L.E., and V.C. Mazurak
- Interactions of linoleic and alpha-linolenic acids in the development of fatty acid alterations in cystic fibrosis, Katrangi, W., J. Lawrenz, A.C. Seegmiller, and M. Laposata
- Plasticity of mouse brain docosahexaenoic acid: modulation by diet and age, Moriguchi, T., A. Harauma, and N. Salem Jr.
- How selected tissues of lactating Holstein cows respond to dietary polyunsaturated fatty acid supplementation, Hiller, B., J. Angulo, M. Olivera, G. Nuernberg, and K. Nuernberg
- Genetic background and diet impact beef fatty acid composition and stearoyl-CoA desaturase mRNA expression, Costa, A.S.H., M.P. Silva, C.P.M. Alfaia, V.M.R. Pires, C.M.G.A. Fontes, R.J.B. Bessa, and J.A.M. Prates
- Inhibitory effect of *N*-acyl dopamines on IgE-mediated allergic response in RBL-2H3 cells, Yoo, J.-M., E.S. Park, M.R. Kim, and D.-E. Sok
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roles in the creation of these levels of order. In the late 1980s, a diverse series of experimental findings collectively gave rise to the lipid raft hypothesis. Lipid rafts were originally defined as membrane domains, that is, ordered structures created as a consequence of the lateral segregation of sphingolipids and differing from the surrounding membrane in their molecular composition and properties. This definition was subsequently modified to introduce the notion that lipid rafts correspond to membrane areas stabilized by the presence of cholesterol within a liquid-ordered phase. During the past two decades, the concept of lipid rafts has become extremely popular among cell biologists, and these structures have been suggested to be involved in a great variety of cellular functions and biological events. During the same period, however, some groups presented experimental evidence that appeared to contradict the basic tenets that underlie the lipid raft concept. The concept is currently being re-defined, with greater consistency regarding the true nature and role of lipid rafts. In this article we will review the concepts, criticisms, and the novel confirmatory findings relating to the lipid raft hypothesis.

Emerging targets in lipid-based therapy

Tucker, S.C., and K.V. Honn, *Biochem. Pharmacol. 85*:673–688, 2013.

The use of prostaglandins and NSAIDS in the clinic has proven that lipid mediators and their associated pathways make attractive therapeutic targets. When contemplating therapies involving lipid pathways, several basic agents come to mind. There are the enzymes and accessory proteins that lead to the metabolism of lipid substrates, provided through diet or through actions of lipases; the subsequent lipid products; and finally the lipid sensors or receptors. There is abundant evidence that molecules along this lipid continuum can serve as prognostic and diagnostic indicators and are in fact viable therapeutic targets. Furthermore, lipids themselves can be used as therapeutics. Despite this, the vernacular dialog pertaining to "biomarkers" does not routinely include mention of lipids, though this is rapidly changing. Collectively these agents are becoming more appreciated for their respective roles in diverse disease processes from cancer to preterm labor and are receiving their due appreciation after decades of groundwork in the lipid field. By relating examples of disease processes that result from dysfunction along the lipid continuum, as well as examples of lipid therapies and emerging technologies, this review is meant to inspire further reading and discovery.

Lipidomics of Alzheimer's disease: current status

Wood, P.L., *Alzheimer's Res. Ther. 4(1)*:5, doi: 10.1186/alzrt103, 2012.

Alzheimer's disease (AD) is a cognitive disorder with a number of complex neuropathologies, including, but not limited to, neurofibrillary tangles, neuritic plaques, neuronal shrinkage, hypomyelination, neuroinflammation, and cholinergic dysfunction. The role of underlying pathological processes in the evolution of the cholinergic deficit responsible for cognitive decline has not been elucidated. Furthermore, generation of testable hypotheses for defining points of pharmacological intervention in AD are complicated by the large-scale occurrence of older individuals dying with no cognitive impairment despite having a high burden of AD pathology (plaques and tangles). To further complicate these research challenges, there is no animal model that reproduces the combined hallmark neuropathologies of AD. These research limitations have stimulated the application of "omics" technologies in AD research with the goals of defining biologic markers of disease and disease progression and uncovering potential points of pharmacological intervention for the design of AD therapeutics. In the case of sporadic AD, the dominant form of dementia, genomics has revealed that the epsilon 4 allele of apolipoprotein E, a lipid transport/chaperone protein, is a susceptibility factor. This seminal observation points to the importance of lipid dynamics as an area of investigation in AD. In this regard, lipidomics studies have demonstrated that there are major deficits in brain structural glycerophospholipids and sphingolipids, as well as alterations in metabolites of these complex structural lipids, which act as signaling molecules. Peroxisomal dysfunction appears to be a key component of the changes in glycerophospholipid deficits. In this review, lipid alterations and their potential roles in the pathophysiology of AD are discussed.

Impact of genetics and environment on the metabolite composition of maize grain

Skogerson, K., *et al., J. Agric. Food Chem. 58*:3600–3610, 2010.

This study sought to assess genetic and environmental impacts on the metabolite composition of maize grain. Gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) measured 119 identified metabolites including free amino acids, free fatty acids, sugars, organic acids, and other small molecules in a range of hybrids derived from 48 inbred lines crossed against two different tester lines (from the C103 and Iodent heterotic groups) and grown at three locations in Iowa. It was reasoned that expanded metabolite coverage would contribute to a comprehensive evaluation of the grain metabolome, its degree of variability, and, in principle, its relationship to other compositional and agronomic features. The metabolic profiling results established that the small molecule metabolite pool is highly dependent on genotypic variation and that levels of certain metabolite classes may have an inverse genotypic relationship to each other. Different metabolic phenotypes were clearly associated with the two distinct tester populations. Overall, grain from the C103 lines contained higher levels of free fatty acids and organic acids, whereas grain from the Iodent lines were associated with higher levels of amino acids and carbohydrates. In addition, the fold-range of genotype mean values [composed of six samples each (two tester crosses per inbred × three field sites)] for identified metabolites ranged from ~1.5- to 93-fold. Interestingly, some grain metabolites showed a non-normal distribution over the entire corn population, which could, at least in part, be attributed to large differences in metabolite values within specific inbred crosses relative to other inbred sets. This study suggests a potential role for metabolic profiling in assisting the process of selecting elite germplasm in biotechnology development, or marker-assisted breeding.

Influence of precursors on the formation of 3-MCPD and glycidyl esters in a model oil under simulated deodorization conditions

Freudenstein, A., *et al., Eur. J. Lipid Sci. Technol. 115*:286–294, 2013.

To find new ways for reducing the potential of palm oil to form 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters during refining, it is helpful to know more about the influence of different precursors such as diacylglycerols (DAG) and monoacylglycerols (MAG), lecithin, and chlorine-containing compounds. After adding increasing amounts of the different precursors to a model oil obtained by removal of polar compounds from crude palm oil and heating the mixture under standardized conditions to 240°C for 2 h the contents of 3-MCPD and glycidyl esters were analyzed according to the standard procedure of DGF (Deutsche Geschellschaft für Fettwissenschaft) C-VI 18 (10). DAG and MAG were found to increase the potential of palm oil to form 3-MCPD and glycidyl esters, but refined lecithin showed no influence. Sodium chloride as well as tetra-*n*-butylammonium-chloride (TBAC) led to higher contents of the esters. Whereas the addition of TBAC raised the amount of glycidyl esters as well as 3-MCPD esters, sodium chloride largely raised the amount of 3-MCPD esters. An addition of 5 mmol of sodium carbonate/kg model oil spiked with sodium chloride reduced the amount of glycidyl esters almost completely; the 3-MCPD esters were reduced by 50%. About 1 mmol sodium hydrogen carbonate/kg oil reduced both 3-MCPD and glycidyl esters almost completely.

Free radical mediated formation of 3-monochloropropanediol (3-MCPD) fatty acid diesters

Zhang, X., *et al., J. Agric. Food Chem. 61*:2548–2555, 2013.

The present study was conducted to test the hypothesis that a free radical was formed and mediated the formation of 3-monochloropropanediol (3-MCPD) fatty acid diesters, a group of food contaminants, from diacylglycerols at high temperature under a low-moisture condition for the first time. The presence of free radicals in a vegetable oil kept at 120°C for 20 min was demonstrated using an electron spin resonance (ESR) spectroscopy examination with 5,5-dimethylpyrroline-*N*-oxide (DMPO) as the spin trap agent. ESR investigation also showed an association between thermal treatment degree and the concentration of free radicals. A Fourier transform infrared spectroscopy (FT-IR) analysis of *sn*-1,2-stearoylglycerol (DSG) at 25°C and 120°C suggested the possible involvement of an ester carbonyl group in forming 3-MCPD diesters. On the basis of these results, a novel free radical-mediated chemical mechanism was proposed for 3-MCPD diester formation. Furthermore, a quadrupole-time of flight (Q-TOF) MS/MS investigation was performed and detected the DMPO

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Mintec

STATISTICAL ANALYSIS FROM MINTEC

James Hutchings at Mintec

During 2012, palm oil prices fell as a good supply in Southeast Asia was met with relatively subdued export demand. In comparison, rapeseed and soybean oil prices have remained more stable (Fig. 1) Production of palm oil in Indonesia and Malaysia has risen significantly in recent years, and world production in 2012/13 is currently forecast at 53.3 million metric tons (MMT), up 5% year-on-year.

Soybean oil world closing stocks in 2012/13 are forecast to fall 12% year-on-year to 3.3 MMTas consumption outstrips production. World soybean oil production is forecast at 43.2 MMT, up 2% year-on-year whereas consumption is forecast to rise 4% to 43.4 MMT, largely driven by China.

World estimates for rapeseed production were recently revised up to 59.3 MMT for 2012/13, but this is still down 4% compared to 2011/12. Falling year-on-year rapeseed production has also led to an expected fall in rapeseed oil production, forecast at 23.5 MMT, down 3% year-on-year. Rapeseed oil exports from Canada, the world's top exporter, are set to fall 7% in 2012/13 owing to expectations that rapeseed plantings will fall 10% in favor of more lucrative crops. This has kept rapeseed oil prices firm and increased the premium that rapeseed oil traditionally enjoys over soybean oil (Fig. 2).

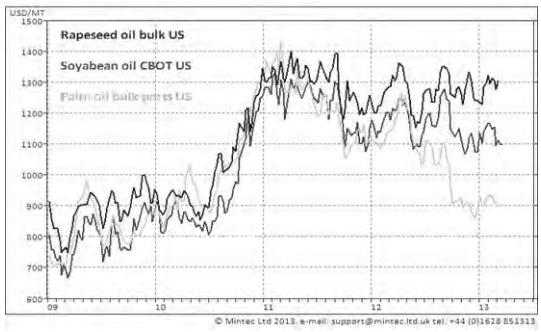

FIG. 1. US prices, 2009–2013 for palm oil, soybean oil, and rapeseed oil. USD/MT, US dollars/metric ton.

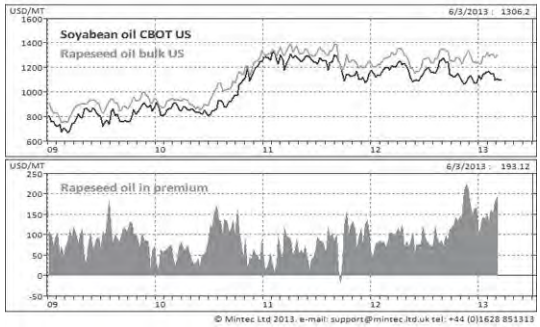

FIG. 2. USD/MT, US dollars/metric ton.

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How many mass spectrometers are enough?
Adventures in multiple parallel mass spectrometry

William Craig Byrdwell

Analysis of lipid mixtures has always been a complicated undertaking owing to the variety of lipid classes that may be present—fatty acids (FA), triacylglycerols (TAG), phospholipids (PL), sterols (ST), and others—and the large number of molecules within each class. The variety of combinations of FA with different chain lengths and different degrees of unsaturation (i.e., molecular species) that can be present in each class is extensive, which is further complicated by the different combinations of how they are arranged on the glycerol backbone (for TAG and glycero-PL) to give different regioisomers, as well as different double bond isomers (e.g., ω-3 vs. ω-6, etc.).

- **Using two or more mass spectrometers in parallel is not impractical or expensive.**
- **Using multiple ionization methods reduces uncertainty and provides more and complementary information in less time.**
- **New methods allow analysts time to interpret data and do other high-value tasks, instead of wet chemistry.**

LC-MS IS IDEALLY SUITED TO UNRAVELING THE COMPLEXITY OF LIPIDS

Although identifying all of these possible combinations is a daunting task, technological advances have risen to the challenge and produced new generations of liquid chromatography-mass spectrometry (LC-MS) instruments and software that allow detailed lipid analysis with greater accuracy and at lower levels than ever. Advances in liquid chromatography instruments as well as column technologies provide an incredible array of options to the lipid chemist. The rise of ultra-high performance liquid chromatography (UHPLC) and the development of sub-2 μm column packings make detection of trace amounts of lipid components more feasible than ever.

Furthermore, recent generations of mass spectrometers that combine conventional mass analyzers (quadrupole, ion trap, time of flight) into new, powerful hybrid configurations (q-trap, q-TOF, etc.) allow detailed structural analyses to be accomplished that were never before possible. Even after all of these advancements, though, no single LC-MS approach is ideal for all lipids, so compromises and trade-offs must still be made.

Atmospheric pressure ionization options. At the nexus between the liquid chromatograph, with its condensed mobile phase, and the mass spectrometer, which usually requires molecules be in a gaseous state for mass analysis, lies the atmospheric pressure ionization (API) interface. Three API interfaces have risen above all others for routine use and are commercially available from most manufacturers. These are (i) atmospheric pressure chemical ionization (APCI), in which atmospheric gases or mobile phase molecules act as chemical reagents to ionize eluted molecules as they pass by a corona needle at high voltage,(ii) electrospray ionization (ESI), in which the API interface acts as an electrochemical cell maintained at high voltage to gently impart a charge (often with addition of an electrolyte to form an adduct), and (iii) atmospheric pressure photoionization (APPI), in which medium-energy photons (~10 eV) from a krypton lamp (sometimes xenon or argon) ionize a molecule, often with the help of a dopant that absorbs the light and ionizes more efficiently than the analyte and, once ionized, transfers the charge to the analyte.

Due to the different mechanisms of ionization and inherently different ionization efficiencies for different classes of molecules,

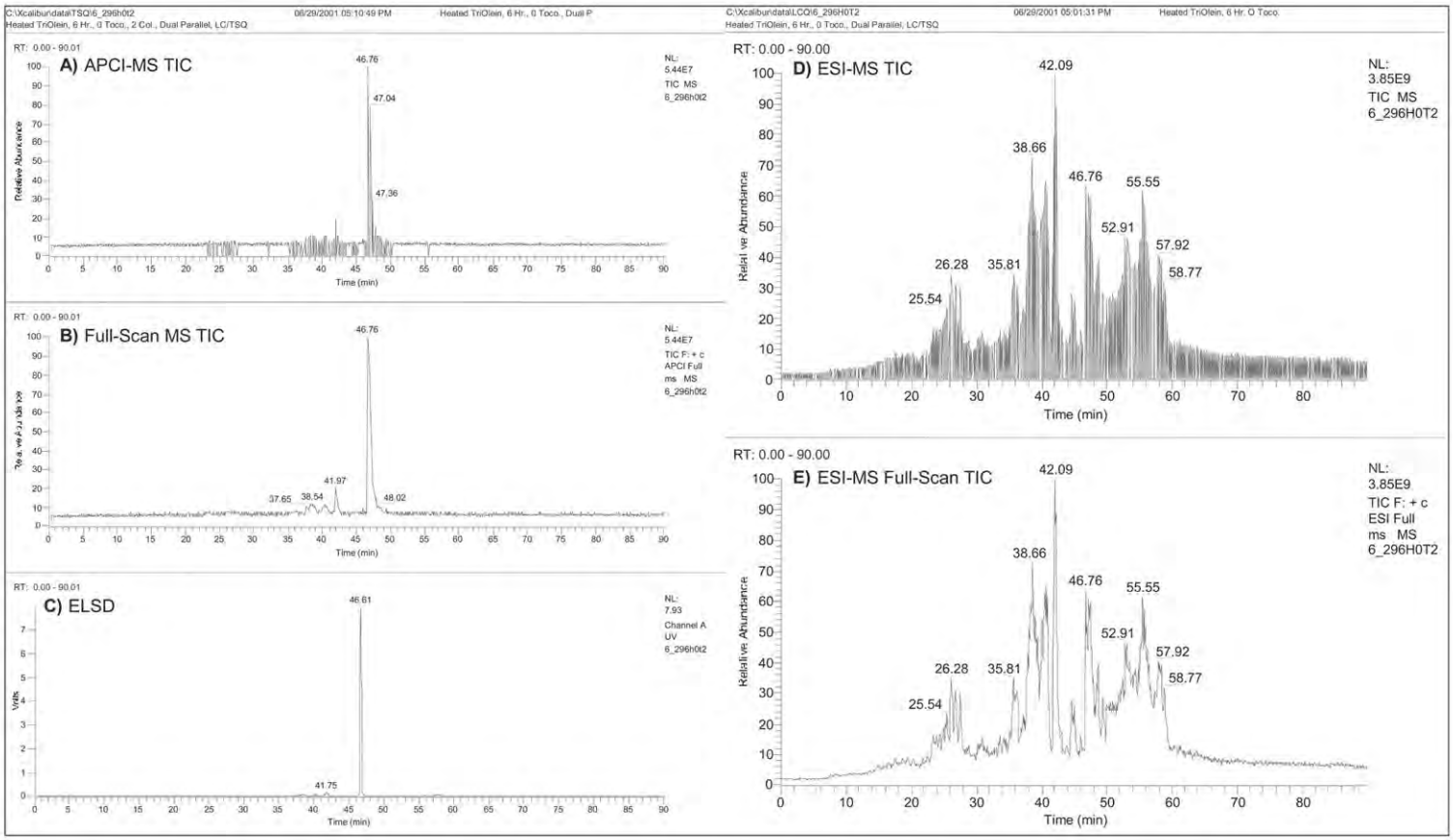

FIG. 1. *Dual parallel atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) and electrospray ionization (ESI)-MS for heated triolein and its oxidation products. (A) APCI-MS with auto-MS/MS; (B) extracted ion chromatography (EIC) of APCI-MS full scans; (C) evaporative light-scattering detector (ELSD); (D) ESI-MS with auto-MS/MS; (E) EIC of full-scan ESI-MS. TIC, total ion current.*

selection of the right API technique is where the choices and compromises begin. Some molecules, such as ST, respond very well to APCI-MS but give virtually no signal by ESI-MS, whereas others, such as PL, are ideally suited to ESI-MS but yield inadequate structural information by APCI-MS. Thus, analysis of some classes of molecules may be sacrificed for the benefit of the analysis of others. This article is about overcoming the drawbacks of any one ionization type to obtain the maximum amount of information possible from MS for every lipid class present.

In our early reports on the unique sphingolipids in the human eye lens(1), we ran samples using LC-APCI-MS and then re-ran them using LC-ESI-MS, to obtain the complementary information that these API techniques produced. ESI-MS gave intact molecules and adducts that provided molecular weight information, but ESI-MS/MS of phosphocholine-containing PL often produced only the phosphocholine head group ion, with little or no information about the backbone and fatty amide chain (or fatty acyl chains for phosphatidylcholines), whereas APCI-MS gave more structural information but much lower abundances derived from the intact molecule. Thus, by obtaining both sets of information, a full picture of the structural characteristics of the molecules could be produced.

Now that API techniques have become the default methods for analysis of many classes of molecules, numerous reports fill the literature in which comparisons are made between sequential runs from complementary API techniques. A database search of "mass spectrometry" and "APPI" and "APCI" returns 100 citations, a search of "mass spectrometry" and "APPI" and "ESI" returns 102 citations, and a search of "mass spectrometry" and "APCI" and "ESI" returns 450 citations! Including APPI, APCI, and ESI together produces 64 citations, but only four seem to be applications to lipids or TAG (found by including "lipid*" or "triacylglycerol*" or "triglyceride*" as keywords). As an increasing number of researchers are finding, it is hard to overestimate the benefit of having two independent streams of MS data to allow conclusive identification of molecular species, especially those at low levels, and those for which the LC separation is less than perfect.

DUAL PARALLEL MS

After our initial report (1) in 1997, it seemed self-evident that it would be much better to obtain these data simultaneously instead

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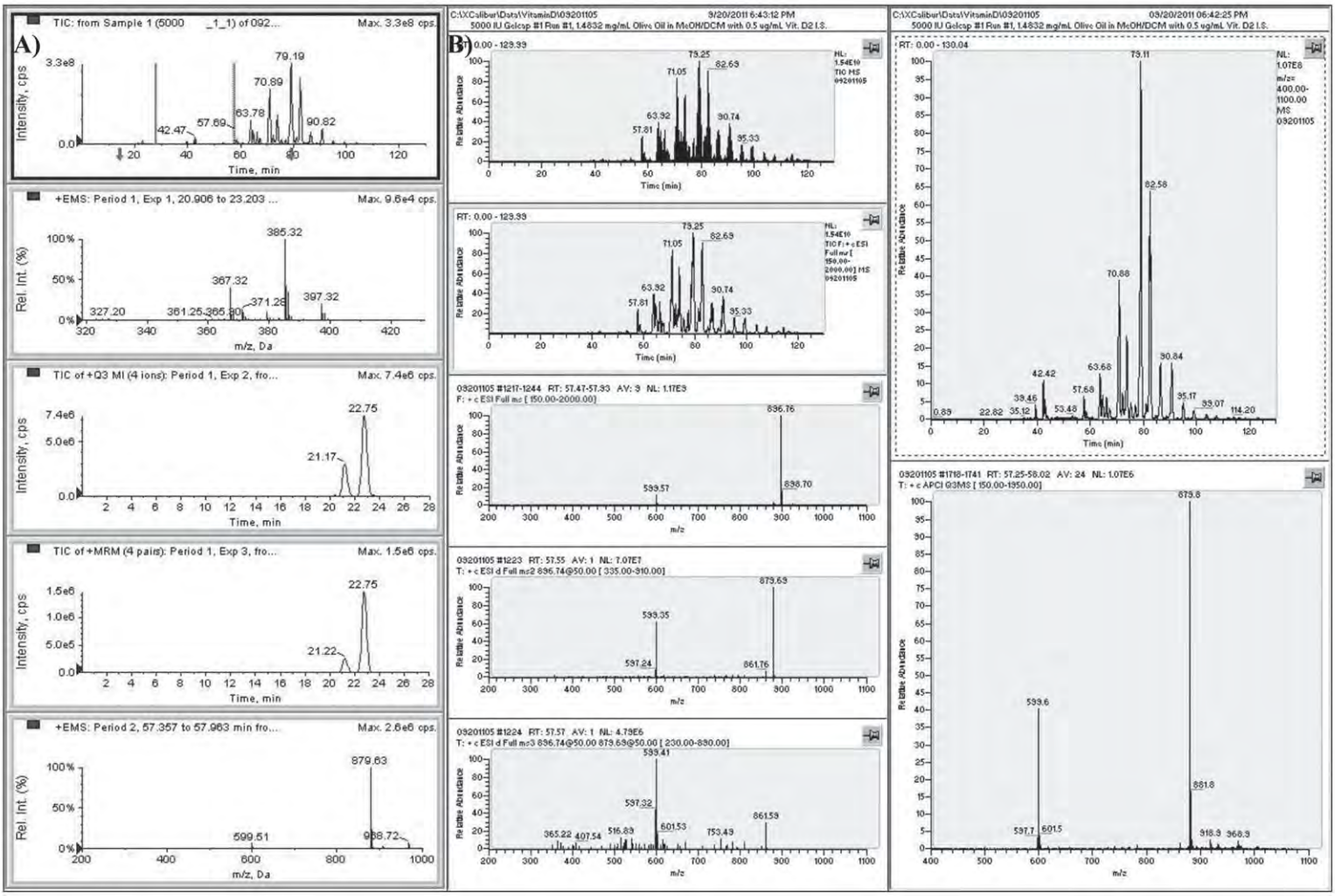

FIG. 2. *Data from triple parallel mass spectrometry analysis of olive oil-filled 5,000 international units (125 µg) vitamin D3 dietary supplements diluted to 100 mL. (A) APCI-MS with selected ion monitoring (SIM) and selected reaction monitoring (SRM) on QTrap 5500 hybrid mass spectrometer; (B) ESI-MS with auto-MS^3 on LCQ Deca XP ion trap mass spectrometer; (C) APCI-MS on TSQ 7000 tandem sector quadrupole mass spectrometer. For other abbreviations see Figure 1.*

of sequentially, so the time, solvents, and resources required for separate runs could be reduced. The next year we published our first report of the use of "dual parallel MS" for analysis of phospholipids (2). Of course, that report from 1998 now appears crude by comparison, since it pre-dated the auto-MS/MS (data-dependent acquisition, DDA) that we now take for granted in all modern MS data acquisition software. The instrument procedures for that early work had to be written manually using the instrument command language, causing the cycle times to be much slower than would be acceptable on a modern instrument. Although crude, those data effectively demonstrated that the dual parallel MS approach was beneficial and that such experiments could be accomplished using simple off-the-shelf components, without the need for extensive prototyping (a figure showing the first phospholipids analysis using atmospheric pressure chemical ionization-mass spectrometry and electrospray ionization simultaneously is available in the supplement to the digital edition, which can be accessed by logging in to read the May 2013 issue at www.aocs.org/login). Splitting of the effluent stream was accomplished using simple tees, with the flow to each instrument mainly dictated by the length and internal diameter of the capillary tubing used for each instrument. To be thorough, data from an ultraviolet (UV) detector and an evaporative light scattering detector (ELSD) were also acquired.

Since that initial report, we have used multiple ionization techniques in parallel whenever possible. Applications of dual parallel MS up to 2005 were presented in the AOCS Press book *Modern Method for Lipid Analysis* (3). One of the best and most cited examples was the use of dual parallel MS for analysis of TAG and TAG oxidation products (TAGOX). Those data, pictured in Figure 1 (page 313), dramatically demonstrate the benefit of ESI-MS of ammonium adducts for TAGOX analysis. TAGOX responded much more sensitively to ESI-MS than APCI-MS, while the ELSD showed that APCI-MS discriminated between classes less than ESI-MS. At the same time, APCI-MS provided valuable fragments

Unique parallel mass spectrometry methods for vitamin D and triacylglycerol analysis

Potential links between vitamin D deficiency and numerous health problems beyond bone health have been identified recently. Awareness of the importance of maintaining adequate vitamin D levels is increasing among consumers and scientists alike.

Existing methods for vitamin D analysis are very labor intensive and time consuming, and there is a great deal of variability in results in the literature. Most methods are similar to those that have been used for decades. They involve saponification—to break down triacylglycerols (TAG), a potential interferent—followed by liquid/liquid extraction, semipreparative chromatography, fraction collection, evaporation and reconstitution, and finally, analytical chromatography. We were quite surprised when we found that, even after all of that sample preparation, including two chromatography steps, some samples, such as processed cheese and orange juice, still showed interfering species that co-eluted with vitamin D in the UV (ultraviolet) chromatograms. In some cases, the co-elution produced nice-looking symmetric peaks that did not belie the underlying troublemaker; but, we were not fooled.

PARALLEL MS FOR VITAMIN D

Based on prior experience, it was only natural to apply dual parallel mass spectrometry (MS) to the analysis of vitamin D in foods. One instrument was dedicated to acquisition of targeted ions for quantification of vitamin D by selected ion monitoring (SIM) atmospheric pressure chemical ionization mass spectrometry (APCI-MS), while a second instrument was used as a "watchdog" for qualitative analysis using full-scan APCI-MS followed by auto-MS/MS. Not only could we see that some samples had peaks that overlapped in the UV chromatograms, we could also identify the masses associated with the interferent(s). For those samples, the UV data could be ignored, and quantification of vitamin D could be accomplished by SIM APCI-MS. For other samples, we had MS data to prove that no interferent was present, so the more sensitive results by UV detection could be trusted. Nowadays, we never trust peaks in UV chromatograms unless there are full-scan MS data to conclusively prove the absence of interfering species.

TRIPLE PARALLEL MS AND MORE

In 2011 we reported the results of a new "dilute-and-shoot" triple parallel MS experiment in which we were able to no longer treat the TAG as interfering species that needed to be broken down and eliminated, but instead were able to perform a holistic analysis of the samples in which both the vitamin D and the composition of the TAG in the bulk oil (rice bran oil in that case) could be determined (*Anal. Bioanal. Chem. 401*:3317–3334). Typical data from three mass spectrometers in a dilute-and-shoot triple parallel mass spectrometer experiment applied to a 5,000 IU vitamin D3 dietary supplement in olive oil are shown in Figure 2 (see main text). Virtually all sample preparation was eliminated, and a single chromatographic system was used for separation of vitamin D2 and D3, as well as pre-vitamin D2 and pre-vitamin D3, if present.

We have now added the capability to perform atmospheric pressure photoionization (APPI)-MS on either of two instruments, in addition to high- and low-sensitivity APCI-MS and electrospray ionization-MS, for quadruple parallel MS, as well as the corona charged aerosol detector, evaporative light-scattering detector, and UV data, for seven detectors overall. We are working to expand the applicability of the dilute-and-shoot approach to other samples.

for structure elucidation, although unusual fragmentation pathways for TAGOX required complementary ESI-MS/MS data to avoid potential misinterpretation.

Such approaches can be implemented by practically any determined analytical chemist, especially after upgrading an instrument in a laboratory. An older instrument that is being replaced can be pressed into service as an auxiliary detector, while at the same time taking advantage of the sensitivity and scan functions available on a newer instrument. Although it is not practical to buy two new mass spectrometers to implement a dual parallel MS approach, it can easily be accomplished during the natural evolution and upgrade of a laboratory over time. Furthermore, used instruments are available at such reasonable cost that the ability to incorporate an additional mass spectrometer is no longer a serious obstacle.

TAKING IT TO THE NEXT LEVEL(S)

In May 2011 in the AOCS Press book *Extreme Chromatography* (4) and in an article published later that year (5), the bar was raised to the next higher level as the first "triple parallel MS" experiment was reported. Typical data for an olive oil-filled dietary supplement containing 5,000 international units (IU) (=125 μg) of vitamin D3 are shown in Figure 2. Previous examples showed the results of the analysis of supplements containing rice bran oil (5) and sunflower oil (4). Vitamin D3 present in dietary supplements at low levels (limit of detection, ~25–90 ng/mL) (5) was analyzed using APCI-MS and UV detection, but the composition of the oil in which the vitamin was dissolved was also determined. Unfortunately, the TAG in the oil overwhelmed the mass analyzer of the newest and most sensitive instrument and led to incorrect isotope ratios and incorrect masses due to improper centroiding (converting a peak profile to a single line at the center of mass). However, an older, less sensitive instrument was ideal for TAG analysis and gave reliable results similar to those obtained in preceding years. Thus, we used high-sensitivity APCI-MS for vitamin D and lower-sensitivity APCI-MS for the bulk oil to accomplish a holistic

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analysis of the supplements. We obtained ESI-MS results simultaneously in parallel that were similar to those obtained in the past, which again provided a valuable complement to the APCI-MS data. UV (single channel and scans), ELSD, and a corona charged aerosol detector (CAD) were also used, for six detectors, overall. Was that enough? Not yet.

We are currently preparing a report on "quadruple parallel MS," using a different combination of instruments. We have added the capability to perform APPI-MS, in addition to high- and low-sensitivity APCI-MS and ESI-MS, having exchanged the instrument on which high-sensitivity APCI-MS was conducted, and adding a hybrid instrument with a higher mass range than the one used previously. We also used an upgraded corona CAD, as well as the ELSD and UV detector, for seven detectors overall. A contact closure distribution panel built from speaker wire and low-cost switches allows different LC systems and MS instruments and other detectors to be reconfigured into a variety of experiments, with the flips of a few switches (photo available in the supplement to the digital edition, which can be accessed by logging in to read the May 2013 issue at www.aocs.org/login).

TIPS AND TRICKS OF THE TRADE

These experiments have allowed us to draw some conclusions about the potential and reality of using multiple complementary mass spectrometers in parallel and to make suggestions regarding their use:

1. Multiple parallel MS can be accomplished by any determined analyst with more than one instrument at his/her disposal using simple off-the-shelf components.
2. Using instruments in parallel saves time, labor, and resources compared to performing sequential runs using different ionization methods and eliminates uncertainty due to run-to-run variability between chromatographic runs.
3. Older instruments can provide a valuable contribution to multiple parallel MS experiments as auxiliary detectors and should be kept in service as long as possible; also, used instruments are very affordable.
4. APCI, APPI, and ESI provide different and complementary structural information on various classes of lipid molecules.
5. Conventional two-dimensional detectors (UV, ELSD, corona CAD) are easily incorporated into multiple parallel MS experiments, and UV is more sensitive than APCI-MS for some classes.
6. Conventional analytical-scale HPLC is more suited to parallel MS than UHPLC because it provides enough flow to split among multiple instruments, peak widths are more compatible with scanning speeds of older instruments, and it allows more DDA experiments across a peak.
7. Some ionization types discriminate between classes more than others (especially ESI), while others show noticeable differences between molecular species within classes.
8. Even within the same type of API source, sources from various manufacturers behave differently and give different signal-to-noise ratios, and some brands discriminate between molecular species more than others.
9. It is helpful to synchronize the system clocks on multiple instruments to minimize minor differences in times shown in figures.
10. It is beneficial to show the time in data headers in figures to show that data were obtained in parallel.

We can finally ask: "Are four mass spectrometers in parallel enough?" The experiments described above have all used one liquid chromatograph with two to four mass spectrometers (LC1/MS2 to LC1/MS4). However, we have also used two liquid chromatographs, with one mass spectrometer attached to each chromatograph, in an LC2/MS2 column-switching experiment in which both polar and nonpolar lipids were simultaneously analyzed on two different LC-MS systems from one injection (6). It is easy to imagine the possibility that APCI, APPI, and ESI could each be used in parallel for detection of the effluent from each of the two LC systems, in an LC2/MS6 experiment (plus UV, ELSD, and CAD, of course). For me, that would probably be "enough"! Or do we need high- and low- sensitivity APCI-MS on one or both? LC2/MS8?

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Fat content determination in animal feed products using a multi-vessel, software-controlled supercritical fluid extraction system

Rui Chen, Jeff Wright, and Tom Phillips

Food processors, animal feed manufacturers, and governmental agencies alike need a fast and reliable measurement for fat content they can use to optimize processes, label food products accurately, and ensure regulatory compliance. Fat content is typically measured using various solvent extraction techniques, including Soxhlet and accelerated solvent extraction (ASE), followed by gravimetric analysis. Typical solvents used include hexane and ether.

- **Supercritical fluid extraction (SFE) can be used as an alternative extraction technique to accelerate solvent extraction (ASE) for fat determination in animal feed products and has the potential to eliminate hydrolysis steps associated with fat analysis.**
- **A software-controlled extraction process offers superior accuracy and precision for fat extraction and ensuing analysis.**
- **Other advantages include a positive impact on environmental sustainability and substantial cost savings.**

Recent attempts have been made to remove fat from various matrices, including oilseeds, meat, dairy products, snack foods, cereals and baked goods, using supercritical fluid extraction (SFE). The low polarity of supercritical CO_2 (SC-CO_2) makes it an ideal solvent for fat. Furthermore, its solvation power can be tuned by varying the pressure and temperature and/or mixing it with more polar organic solvents to achieve the desired selectivity. Other advantages of SFE include the following: (i) Carbon dioxide is relatively nontoxic, noncombustible, and inexpensive; (ii) extracts are easy to recover; and (iii) the need to consume and dispose of organic solvents is reduced.

In the following, we present a case study on fat determination in a variety of animal feed products using software-controlled SFE. The Waters MV-10 ASFE™ System (Fig. 1, page 318) that we used

CONTINUED ON NEXT PAGE

FIG. 1. *Photograph of a Waters MV-10 ASFE™ System.*

TABLE 1. Key extraction parameters of ASE and SFE experiments[a]

ASE		SFE	
Temperature (°C)	125	Temperature (°C)	40
Pressure (psi)	1,000	Pressure (psi)	3,625
Extraction solvent	Hexane	Co-solvent	Methanol
Oven heat up time (min)	5	Co-solvent%	10%
Oven heat up cycle	2	Flow rate (mL/min)	8
Static time (min)	3	Software	ChromScope™
Static cycle	2	Total run time (min)	25
Flush volume	60%		
Purge time (min)	1		
Purge cycle	2		
Total run time (min)	18		

[a]Abbreviations: ASE, accelerated solvent extraction; SFE, supercritical fluid extraction.

consists of a fluid delivery module, an oven that houses up to 10 extraction vessels ranging from 5 to 25 mL, a backpressure regulator (BPR), and a collection module with 12 collection vessels. The system is controlled by ChromScope Sample Prep™ software.

A total of 15 commercially available animal feeds were used as received unless noted otherwise. To gauge the general applicability of SFE in fat analysis, a conscious effort was made to ensure the sample diversity of both the product form and the animals for which the feed was intended. For each SFE extraction, approximately 3 g of finely ground animal feed was loaded into a 5 mL extraction vessel. For each ASE experiment, approximately 3 g of finely ground animal feed was loaded into a 10 mL extraction cell. Detailed extraction conditions for both ASE and SFE are listed in Table 1. The extracted fat content was collected into a preweighed scintillation vial. After the solvent in the extract was removed using a Zymark Turbovap LV, the vial was reweighed. The weight gain was considered the fat content in the sample. It has to be pointed out that an excessive extraction time (25 min) was used for all SFE experiments to ensure the thoroughness of the extraction. In practice, it was noted that the fat started to elute out in less than 5 min. A separate time course study indicated that the optimal extraction time for fat analysis can be shortened to less than 10 min.

The fat analyses of 15 different animal feed products are summarized in Table 2. It is noteworthy that samples 8 and 9

TABLE 2. Fat content of 15 different animal feed products[a]

Vial no.	Type	Target animal	Claim (%)	Fat by ASE (%)	Fat by SFE (%)
1	Pellet	Equine	12.000	12.950	12.209
2	Textured	Cattle	2.000	4.300	2.093
3	Pellet	Equine	7.000	7.006	8.033
4	Ground	Poultry	8.000	7.945	8.652
5	Hay	Cattle	4.000	3.978	4.227
6	Textured	Multispecies	5.000	4.966	5.840
7	Textured	Equine	2.750	3.447	2.602
8	Dried bakery product	Ingredient	7.000	6.800[a]	7.090
9	Pellet	Dog	14.000	13.600[a]	14.693
10	Pellet	Sheep	2.000	2.833	2.646
11	Pellet	Lamb	2.000	2.735	2.120
12	Textured	Equine	10.000	9.811	10.070
13	Dried beet pulp	Ingredient	0.250	0.671	0.238
14	Textured	Cattle	3.500	3.589	4.081
15	Pellet	Equine	12.000	12.400	12.265

[a]Samples were hydrolyzed by acid prior to ASE. For abbreviations see Table 1.

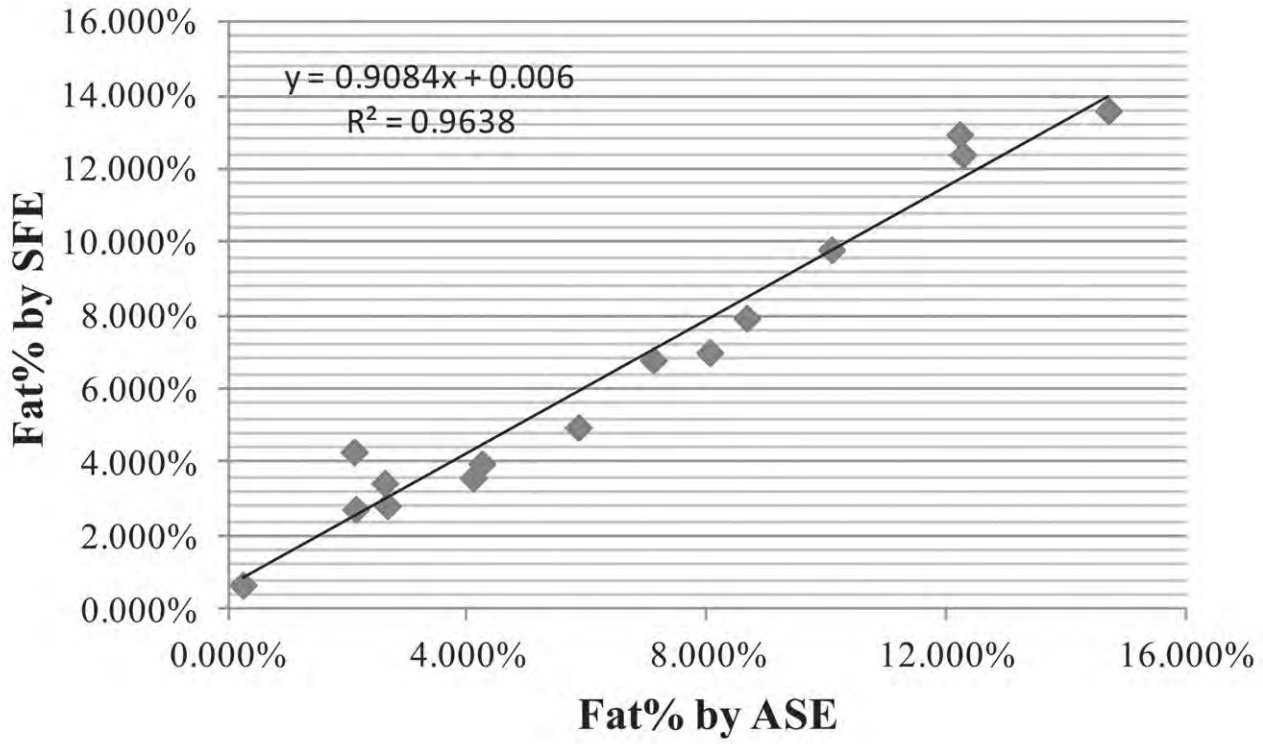

FIG. 2. *Correlation of fat content extracted by supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE).*

were hydrolyzed by acid before ASE. For ASE-based methodology, it is common to include a hydrolysis step before extraction to disintegrate the sample, disrupt the plant cell walls, and liberate the "bound" fat. The hydrolysis step, however, did not seem necessary for the SFE approach. For both samples 8 and 9, the fat content extracted by SFE was slightly higher than that by ASE. This is likely due to the unique properties of SC-CO_2—its gas-like diffusivities and liquid-like solvating strengths enable CO_2 to easily penetrate otherwise difficult matrices, such as glutinous particles in dried bakery product (sample 8), and to dissolve and transport the fat contents.

The correlation of the fat content extracted by SFE and ASE is shown in Figure 2. There is a general agreement between the two extraction techniques, suggesting SFE could become a potential alternative to ASE for fat analysis. The distribution of the Fat% based on the SFE approach is shown in Figure 3. The Fat% ranges from 95% to 132%, with 11 out of 15 samples ranging from 100% to 116% (Table 2), suggesting a good agreement with the label claims.

TABLE 3. Results of fat analysis by SFE from five replicate experiments[a]

Vial no.	Empty vial (g)	Fat + vial (g)	Sample wt. (g)	Fat%
1	24.6961	24.8251	3.150	4.095
2	24.7558	24.8848	3.090	4.175
3	24.6134	24.7424	3.070	4.202
4	24.9672	25.0962	3.220	4.006
5	24.8170	24.9486	3.180	4.138
Mean				4.123
STD				0.0007674
RSD%				1.861

[a]Abbreviations: STD, standard deviation; RSD%, relative standard deviation, percentage. For other abbreviation see Table 1.

A reproducibility analysis of the SFE-based approach for fat analysis is presented in Table 3. The relative standard deviation percentage of the five replicate experiments is less than 2%.

The Waters MV-10 ASFE System is controlled by ChromScope software. After samples are loaded to the extraction vessels,

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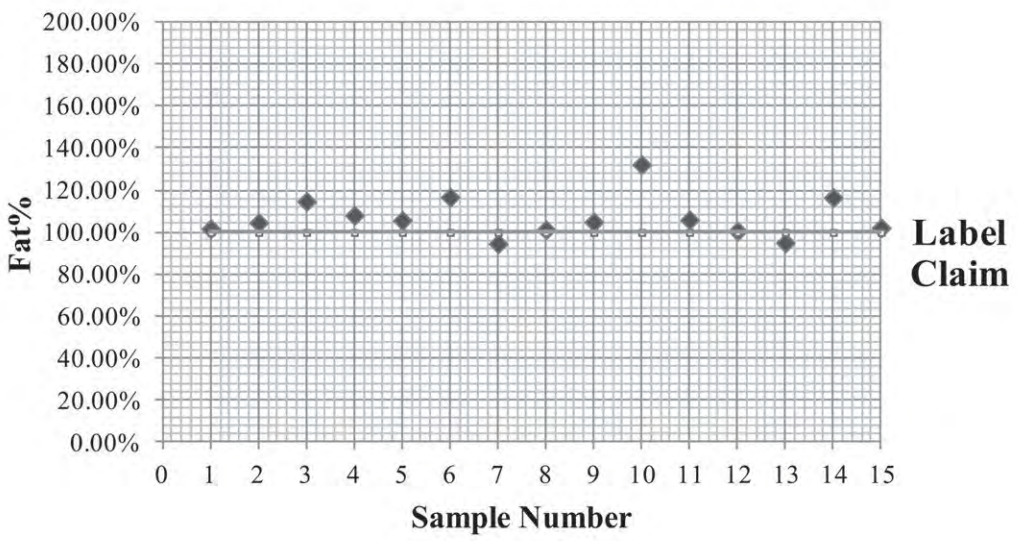

FIG. 3. *Extracted Fat% by SFE vs. label claims for 15 samples. For abbreviation see Figure 2.*

oilseed is approved by AOCS (Am 3-96). Historical reasons for this slow adoption include instrument robustness, level of automation, and to a lesser extent, capital budget constraints in many laboratories. With continuous improvement on instrument design, some of the perceived shortcomings of SFE have been addressed. For example, the Waters MV-10 ASFE System used in this case study features software-controlled BPR for improved accuracy and precision, unattended sequential operation for up to 10 vessels suited for routine analysis of large number of samples, and user-programmable experimental parameters for easy method development and optimization. With escalated emphasis on the "greenness" across many industries, it is time to revisit this somewhat dormant subject. This will require a concerted effort from instrument vendors, research laboratories, academia, and government agencies.

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there is no user intervention required for the extraction; thus, the instrument offers a superior precision for the ensuing analyses.

Here, we demonstrate that SFE can be used as an alternative extraction technique to ASE, which is the industry standard for determining fat content in animal feed. In addition, a software-controlled extraction process offers superior accuracy and precision for the overall analyses. The SFE-based approach also has the potential to eliminate hydrolysis steps that are often required when other extraction techniques are used. Finally, in addition to the positive effect of using a "green" solvent on environmental sustainability, the use of methanol in SFE compared to hexane used in ASE could also result in substantial cost savings.

Despite the successful determination of fat in various matrices, to date, only one SFE-based method for determining oil in

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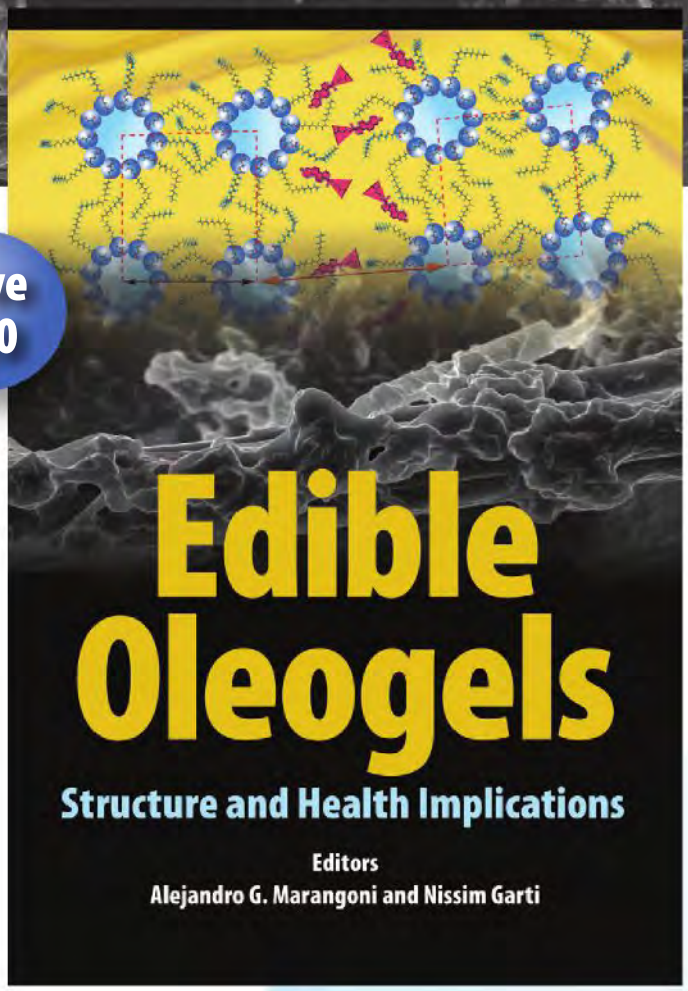

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The rapidly developing Ukrainian oilseeds market

Svetlana Synkovskaya

- Reasons for the rapid expansion of oilseeds in Ukraine
- The major players in the Ukrainian vegetable oils markets
- Factors that will determine market development in the next few years

Oilseeds have experienced impressive gains in sowing areas and yields in Ukraine. During the last 10 years, the areas planted with oilseeds more than doubled—from 3 million hectares to 6.8 million hectares—and all three of the oilseeds most commonly grown in Ukraine (sunflower, rape, and soy) increased their sowing areas.

Why has a country that has a reputation for being a breadbasket shifted toward oilseeds? Here are some reasons:

- High profitability. Farmers understand that, with few exceptions such as winter rapeseeds, oilseeds are less susceptible to weather risks and generate higher profits compared to grains. A look at the last five years reveals a trend: The profitability of oilseeds—especially of sunflower and soybean—increased every year, while the profitability of grains, such as wheat and barley, fell.
- Less state regulation. The government of Ukraine has historically regulated oilseeds less stringently than grains. Because grain-related issues are a matter of national food security, prices for bread are a political issue and are strictly controlled. Indeed, during the last five growing seasons, the grain markets of Russia and Ukraine were a target for numerous state regulations—from export duties, to export quotas, to export bans. Consequently, many farmers and traders view oilseeds as a calm harbor for unpredictable storms of state regulation.
- Galloping development of the crushing industry. The crushing industry has been developing at a rapid pace, and the resulting increase in crushing capacity has led to an increase in production volumes. Today we can divide crushers into two types: (i) big companies that cover the full supply chain (from purchasing, to crushing, to refining, to marketing, to distribution) and (ii) numerous small local crushing factories. Both types are struggling to secure raw material and must compete with other companies to buy oilseeds from farmers. Farmers benefit from this competition, and some of them even get additional support from the larger companies in aspects such as forward purchasing and assistance in securing a seeds supply.

Sunflowerseed is the major oilseed crop in the Ukraine, and areas planted with sunflower occupy nearly 70% of the area devoted to the cultivation of all oilseeds in the country. The high domestic demand for sunflowerseed and its relatively high profitability have resulted in significant expansion of the hectares planted with sunflower (to 4.7 million hectares in 2012). Unfortunately, in spite of scientifically identified optimal spacings of plantings, there has been notable overplanting. This activity has led to problems such as the spread of disease, intensification of pest development, and reduction of soil fertility. Further optimization through crop rotation and planting technologies is one way to solve these problems.

CRUSHING EVOLUTION (INCREASE OF CRUSHING CAPACITIES)

The production and crushing of sunflowerseeds in Ukraine have a regional focus. Figure 1 illustrates the regions with biggest concentration of crushing capacity. The five main

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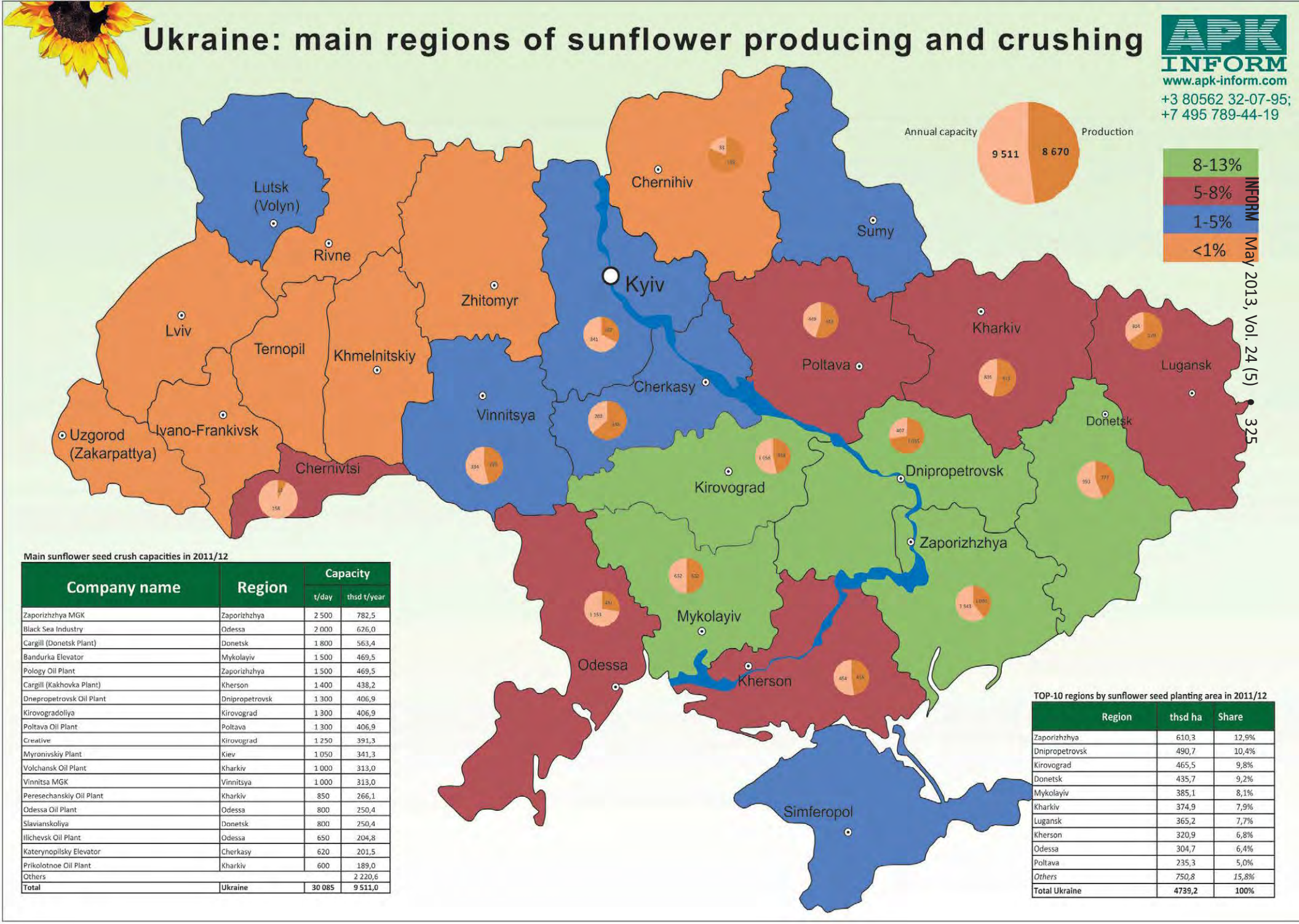

Main sunflower seed crush capacities in 2011/12

Company name	Region	Capacity	
		t/day	thsd t/year
Zaporizhzhya MGK	Zaporizhzhya	2 500	782,5
Black Sea Industry	Odessa	2 000	626,0
Cargill (Donetsk Plant)	Donetsk	1 800	563,4
Bandurka Elevator	Mykolayiv	1 500	469,5
Pology Oil Plant	Zaporizhzhya	1 500	469,5
Cargill (Kakhovka Plant)	Kherson	1 400	438,2
Dnepropetrovsk Oil Plant	Dnipropetrovsk	1 300	406,9
Kirovogradoliya	Kirovograd	1 300	406,9
Poltava Oil Plant	Poltava	1 300	406,9
Creative	Kirovograd	1 250	391,3
Myronivskiy Plant	Kiev	1 050	341,3
Volchansk Oil Plant	Kharkiv	1 000	313,0
Vinnitsa MGK	Vinnitsya	1 000	313,0
Peresechanskiy Oil Plant	Kharkiv	850	266,1
Odessa Oil Plant	Odessa	800	250,4
Slavianskoliya	Donetsk	800	250,4
Ilichevsk Oil Plant	Odessa	650	204,8
Katerynopilsky Elevator	Cherkasy	620	201,5
Prikolotnoe Oil Plant	Kharkiv	600	189,0
Others			2 220,6
Total	**Ukraine**	**30 085**	**9 511,0**

TOP-10 regions by sunflower seed planting area in 2011/12

Region	thsd ha	Share
Zaporizhzhya	610,3	12,9%
Dnipropetrovsk	490,7	10,4%
Kirovograd	465,5	9,8%
Donetsk	435,7	9,2%
Mykolayiv	385,1	8,1%
Kharkiv	374,9	7,9%
Lugansk	365,2	7,7%
Kherson	320,9	6,8%
Odessa	304,7	6,4%
Poltava	235,3	5,0%
Others	*750,8*	*15,8%*
Total Ukraine	**4739,2**	**100%**

FIG. 1. Ukraine: main regions of sunflower producing and crushing.

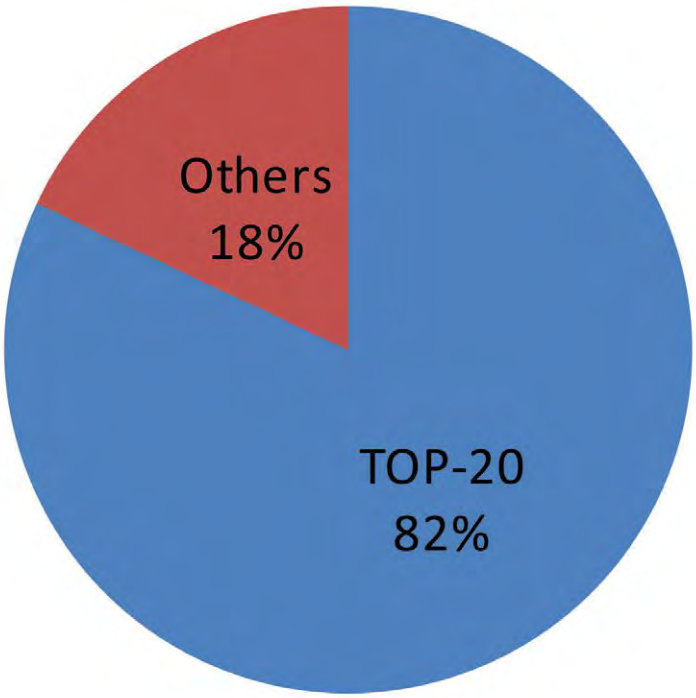

FIG. 2. Distribution of the market shares of sunflower, soybean, and rapeseed oil-producing enterprises in Ukraine. MY Marketing Year.

regions of sunflower production in Ukraine are highlighted in orange. The share of sunflower plantings in these regions represents 8 to 13% of the overall planted areas. Three of these regions have additional logistical advantages, including access to sea ports, which allows oils and meals to be exported at a lower cost. The map also shows that in some regions crushing is more developed than processing. For example, in Zaporizhzhya and Kirovograd, crushing volumes exceed production, which results in strong competition between crushers for raw materials (mostly sunflower).

The situation is quite different for the other main oilseeds crops. Rapeseeds crushing is still underdeveloped, with the majority of rapeseeds being exported without processing. As for soybeans, the crushing industry is just beginning to develop, primarily in the Kirovograd and Kherson areas.

The main players in the Ukrainian vegetable oils markets (traders and crushers) include both domestic and transnational companies such as Bunge, Cargill, Glencore, Kernel, and Creativ (Fig. 2). Although more than 900 enterprises, including small oilmills, produce sunflower oil in Ukraine, the five largest oil-producing plants constitute a third of the market, and the first dozen make up nearly two-thirds of the market. The list of the top 20 covers over 80% of the general production of sunflower oil in the country.

In considering only total production, not individual processing plants, more than half of the production of sunflower oil in 2011/12 MY was controlled by four major agro-industrial associations: Kernel, Cargill, the group "Privat," and Bunge. Each of these market players has its own priorities and strategies for developing the market. For example, Kernel, the group "Privat," and Bunge are not only big exporters, but are also suppliers in the domestic market, where they effectively develop brands of packaged oil and other fat- and oil-based commodities while producing significant additional volumes for export trading. Cargill, on the other hand, is mainly engaged in the production of crude sunflower oil, which is actively exported.

EXPORT AMBITIONS

The Ukrainian processing sector is totally export oriented, so export demand will remain a dominating factor of the sector's development in the mid-term. Consequently, Ukrainian markets pricing strongly depends on global market prices. A strong oilseeds crop during the last several seasons has resulted in impressive crushing volumes. In 2012, crushing in Ukraine hit several monthly records. Yet, while exports dramatically exceed domestic consumption, domestic vegetable oil consumption (more than 15 kg per person per year) is still above the (Food and Agriculture Organization (FAO) recommended value of 13.2 kg per year.

In 2011/12 MY Ukrainian sunflower oil was exported to 94 countries throughout the world, with the largest quantity going to India. In 2011/12 MY, this country was the recipient of 34% of the exported oil, or 1.12 million metric tons (MMT), up from 27%, or 0.7 MMT, during the previous season.

Other large-scale importers of Ukrainian sunflower oil in 2011/12 MY included the countries of Middle East and North Africa: Egypt [350,000 metric tons(MT)], Iran (233,000MT), Algeria (220,000 MT), and Turkey (206,000 MT).

In the 2011/12 MY the top 10 producers of sunflower oil in Ukraine processed 80% of the total volume of sunflower oil and sold 74% of the nation's production on the export market.

As a leading agribusiness consulting agency in the Commonwealth of Independent States countries, APK-Inform estimates that, during the past growing season, Ukraine produced 3.74 MMT of sunflower oil, a record rate for the country and a 19% increase over what was produced in 2010/11 MY. During that time, sunflower oil exports increased by 23%, reaching a maximum level of 3.26 MMT.

APK-Inform Agency estimates that sunflower oil production in Ukraine by oil-extracting factories will total 3.55 MMT in 2012/13 MY (down 5% from 2011/12 MY), and export opportunities will reach a level of 3 MMT (down 7%).

What will the ranking of the sunflower oil major producers be in 2012/13 MY? Obviously the season will be interesting, more tightened, and the rating can be essentially changed. If the stated projects for building new oil-extracting factories come on-stream, some of the participants will improve their presence on the market, and there will be new players. If there is a decrease in sunflower seed production volumes next season

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A growing presence in the world market for soybeans

Although farmers have planted soybeans in Ukraine since the 1870s, commercial production of soybeans is relatively new. Average yields did not exceed 0.7–0.9 metric tons per hectare until the late 1990s, when cultivation rates began to increase.

During the past 10 years, both yields and the areas cultivated with soybeans in Ukraine have increased dramatically. By 2009/10 MY, Ukraine was the 10th-largest producer of soybeans in the world. In 2011/12 MY, soybean production Ukraine reached a record level of 2.2 million metric tons (MMT), up 35% from the previous season's index; and planted areas and yield also achieved maximum levels that totaled 1.13 million hectares and 2.04 metric tons/hectare—an annual increase of 5% and 26%, respectively.

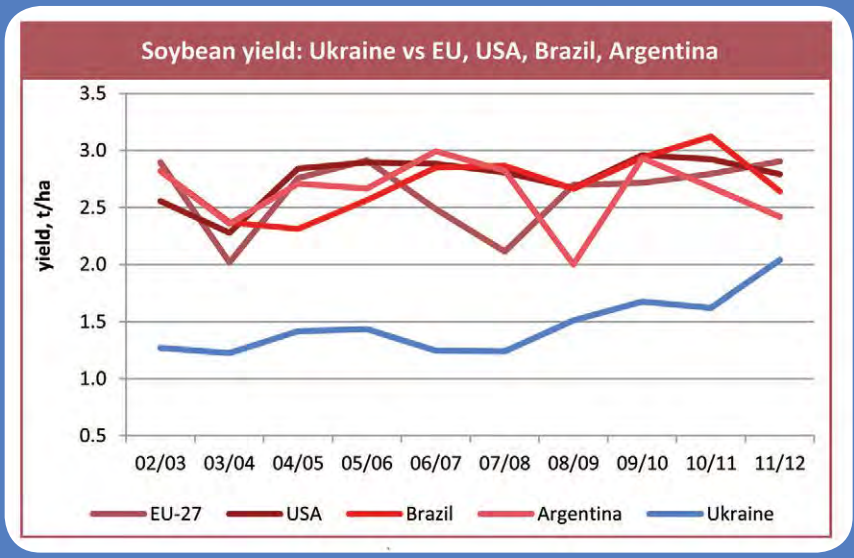
Today, Ukraine is the eighth-largest producer of soybeans, having surpassed the EU-27, Bolivia, and Uruguay—all of which have had more years of experience growing the oilseed. However, when comparing commercial soybean production in Ukraine with other countries, it is important to note that commercial production in Ukraine is still actively developing and has not achieved technical efficiency yet. Thus, while the yield indices of Ukrainian soybeans exceed those of India (by nearly 0,7 t/ha during recent three seasons), Russia (by 0,5 t/ha), and China (up 0.6 t/ha), they are lower than those of the top three growers (1.12 MT/ha lower than those in Brazil, 1.1 MT/ha lower than those in the United States, and 1 MT/ha lower than those in the EU-27 (Fig. 3)

In spite of rapid growth in soybean production, the soybean processing industry in Ukraine has been relatively slow to develop. There are a variety of reasons for this. One is that sunflower seeds have traditionally been the rawmaterial for the fat-and-oil industry in Ukraine, while soybeans and rapeseed have historically been export-oriented commodities. The export potential of soybeans in the Ukraine is expected to reach 1.9 MMT in 2014/15—500,000 metric tons (MT), or 36%, higher than it was in 2011/12 MY.

Meanwhile, soybean crushing volumes remain modest. APK-Inform Agency estimates that in 2012 the Ukrainian enterprises processed nearly 300,000MT of soybean.

However, we forecast that by 2014/15 MY Ukraine will increase soybean production volumes to 3.1 MMT. This will provide the base needed to increase domestic oilseed processing as well as export potential. At the same time, we predict that exports will continue to exceed domestic consumption with respect to volumes and specific weight in the general distribution, with the ratio to total 62% to 38%, respectively.

We expect that Ukraine's presence in the world markets for soybean oil and soybean meal will continue to expand in the future as soybean production increases and domestic processing evolves.



Abbreviation: t/ha, metric tons per hectare. Source: USDA, State Statistics Service of Ukraine

TABLE 1. Ukraine crushing capacity (MMT) and volumes (MMT) of oilseeds crushing (including forecast) for 2008–2014[a]

	08/09	09/10	10/11	11/12	12/13 F	13/14 F
Total crushing capacity	7.935	8.871	10.101	10.723	12.431	14.587
including: sunflower crushing capacity	7.218	8.133	9.309	9.817	11.369	13.497
Volumes of oilseeds crushed	6.717	7.749	7.898	9.443	9.170	
including: volumes of sunflower crushed	6.227	7.266	7.482	8.943	8.450	
Vegetable oils production	2.733	3.190	3.222	3.849	3.676	

[a] Source: APK-Inform Agency Forecast. Abbreviation: MMT, million metric tons.

the oil-extracting factories will be working at less than capacity. So there is high attention for the other raw-materials such as soybeans and rapeseed.

GOOD PROSPECTS IN SPITE OF RISKS

Three factors will determine market development in the next few years:

1. Increase of crushing capacities. Investors are optimistic about Ukrainian crushing industry prospects. This year several big local players announced plans to invest in increased crushing and refinery capacity. APK-Inform Agency analysts estimate that in 2011/12 MY the general processing capacities of sunflowerseed in Ukraine totaled nearly 10.7 MMT per MY. With modernization of oil-producing plants, the launch of new product lines, and construction of new facilities, the nominal processing capacities of the industry could reach the level of about 15 MMT during the current marketing year (Table 1, page 327).

2. Restriction of sunflower areas. In March 2012, a new land-use law that includes more strict control over crop-rotation took effect, and government officials began to make statements about sunflower crops being dangerous to soil. In reality, there is no clear mechanism to prevent farmers from planting sunflower in the same field more often than is recommended. So, in spite of rumors, we do not think the new law will seriously impact the sunflower market this season. In the same time, the share of land devoted to the production of sunflower relative to other oilseeds has decreased during the last five years from 94% to 71%, while rapeseed increased its share from 3% to 13%, and soybeans increased from 4% to 17%. This growth in soybean production has come at the expense of rapeseed, for which the growing area has been reduced.

3. Integration and consolidation. Each year, the share of small crushing plants decreases while the share of the largest players increases. If this trend continues, we predict that in next 2–3 years the top 10 crushers could control about 80% of the crushing and export markets for Ukrainian oilseeds.

Svetlana Synkovskaya [Marketing Director] at APK-Inform, a leading agribusiness consulting agency in the Commonwealth of Independent States countries. She can be contacted at marketing@apk-inform.com.

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Lipid Oxidation
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Amy Logan, Uwe Nienaber, and Xiangqing (Shawn) Pan, Editors
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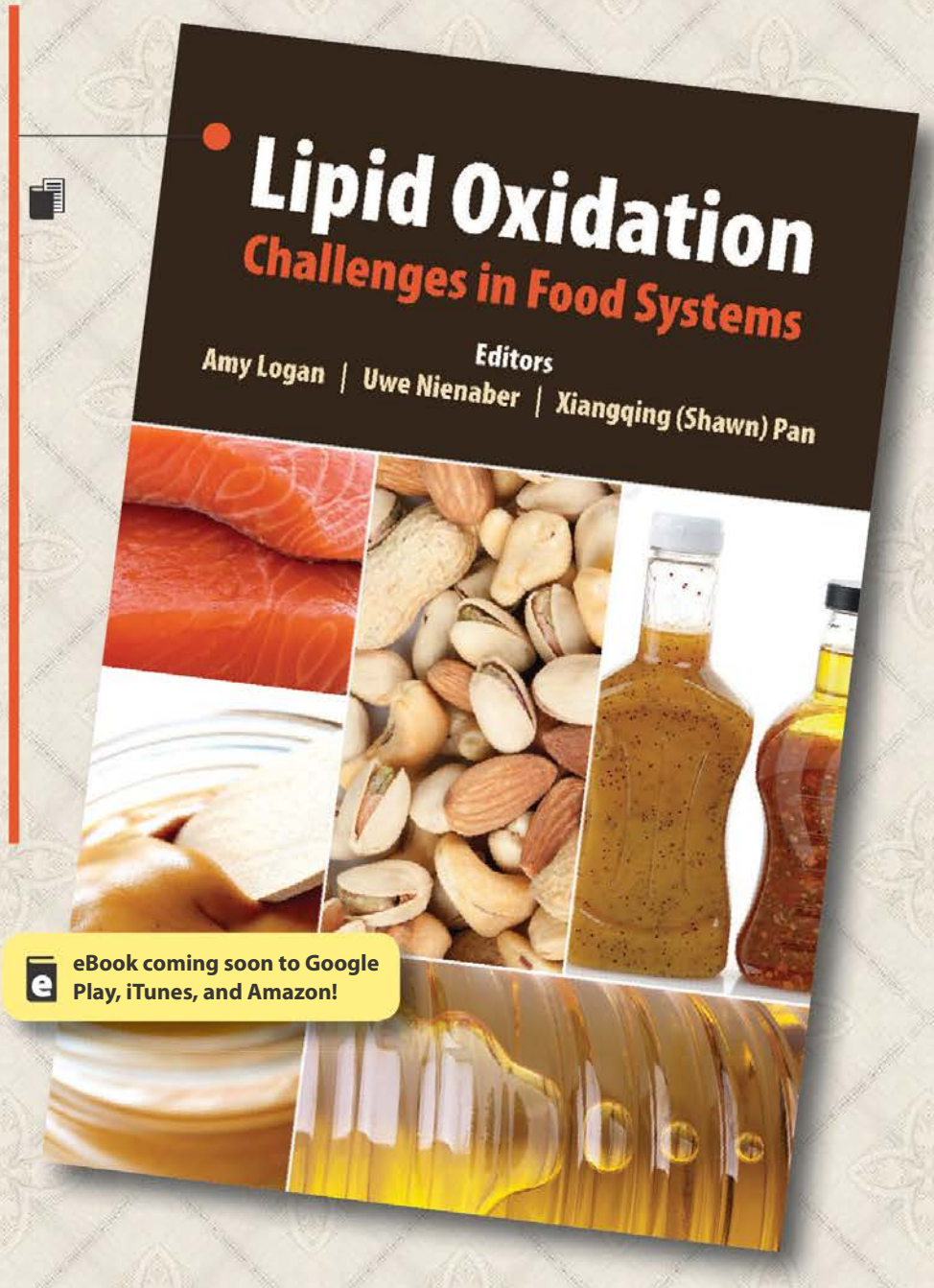
Lipid oxidation in food systems is one of the most important factors which affect food quality, nutrition, safety, color and consumers' acceptance. The control of lipid oxidation remains an ongoing challenge as most foods constitute very complex matrices. Lipids are mostly incorporated as emulsions, and chemical reactions occur at various interfaces throughout the food matrix. Recently, incorporation of healthy lipids into food systems to deliver the desired nutrients is becoming more popular in the food industry. Many food ingredients contain a vast array of components, many of them unknown or constituting diverse or undefined molecular structures making the need in the food industry to develop effective approaches to mitigate lipid oxidation in food systems. This book provides recent perspectives aimed at a better understanding of lipid oxidation mechanisms and strategies to improve the oxidative stability of food systems.



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Chicago's specialty chemical pioneers: Best Foods Co. (1898–2000)

This is the first in a series of historical articles about a number of individual chemists in Chicago who successfully established new specialty chemical businesses during the 1920s, 1930s, and 1940s, many during the Great Depression, when synthetic organic chemistry was in its infancy. These remarkable, entrepreneurial chemical pioneers had several characteristics in common. They were courageous, determined risk-takers who identified what the market needed and worked to fulfill those needs. Their professional careers in chemistry are examined through the specialty chemical companies involved, including Best Foods Co., Emulsol, Witco, Ninol, and Stepan.

Edward A. Knaggs

Chicago spawned a lot more than Al Capone (pictured) and speakeasies in the 1920s, '30s, and '40s. It gave birth to a new specialty chemicals industry that, in turn, gave rise to new products, new processes, new markets, new companies, new jobs, new profits, and developing new technologies.

By 1925, metropolitan Chicago was well on its way to becoming one of the largest industrial cities in the world with the following major industries (listed and ranked according to their economic importance): "slaughtering/meat packing, printing/publishing, foundry/metal working, electrical, clothing, iron/steel making/milling, pipe fittings, food and personal care industries" (1). Many of these Chicago industries employed chemists in support of their operations. Examples included Best Foods Laboratories, Swift & Co. in the stockyards, Armour & Co.'s nearby laboratories, and Universal Oil Products' suburban Riverside facilities providing petroleum refining process research support for the oil industry.

In 1922, giant conglomerate Corn Products Corp.'s Gold Dust subsidiary had its Best Foods consumer products division consolidate its various food and related consumer products operations, and established a relatively large laboratory in Chicago. Its product lines were gradually increased to include Karo Corn Syrup, other syrups, jams, Mazola Corn Oil, Hellmann's Mayonnaise, dressings, margarine, Shinola Shoe Polish, Gold Dust soap products, laundry starches, and others. Because of its size, Best Foods was able to attract a number of well-educated chemists. Some were graduates of the University of Chicago, while others were European immigrants seeking better opportunities in the New World.

By 1933, in the midst of the Great Depression, President-elect Franklin D. Roosevelt proposed a job-saving program, asking employers to retain their employees, putting them on a

CONTINUED ON NEXT PAGE

Specialty chemicals are generally defined as chemical products or intermediates that contribute or impart special physical or chemical features and/or improved product performance properties or attributes to a formulation or consumer product. Typically, the unique features of the somewhat lower-volume specialty chemicals may warrant or command a premium or price advantage that helps to differentiate them from the larger-volume, chemically well-defined, lower margin-priced commodity chemicals.

half-time basis with reduced pay. For necessary cost- and job-saving measures, Best Foods' management decided to follow the president's recommendation. Thus, all employees' work was reduced to half time, with drastically reduced wages. As an incentive to keep its chemists, Best Foods allowed its lab personnel to utilize their laboratory facilities to pursue projects of their own choosing, working on their own free time. Some used this opportunity to develop new and patentable products and processes for themselves (2).

Over the years, Best Foods' laboratories proved to be a training ground for many of its chemists, especially during and right after the Great Depression. Some left the company and established their own specialty chemical companies, while others left to join those who already started new businesses. This group of former Best Foods chemists included Russian immigrant Wolf Kritchevsky, a well-educated dye expert, who left Best Foods in about 1934 to start Rit Products Co. in Chicago, His patents on alkylolamine–fatty acid condensation products (later to be named Ninols) would contribute to his sons starting Ninol Laboratories in Chicago in 1940 (2). Morris B. Katzman and Albert K. Epstein, also working on their own free time, secured a patent on certain fatty amide emulsifying agents.

Albert K. Epstein left Best Foods in 1935 to become president of his new chemical company called Emulsol Corp. Other Best Foods chemists left and joined Emulsol, including Benjamin R. Harris, Morris B. Katzman, Frank J. Cahn, and John J. Morrisroe (2).

INFORMATION

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5. History and timeline of Pilot Chemical Co. www.pilotchemicalco.com (accessed February 2012).
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Katzman and Cahn left Emulsol in 1952 to start Process Chemicals Co. in Los Angeles, California, USA (2,3). John Morrisroe left Emulsol in 1951, invented his SO_2 solvent SO_3 sulfonation process (4), and started Pilot Chemical Co. in Santa Fe Springs, California, in 1952 (5).

Best Foods continued to grow globally as the largest international food giant, constantly undergoing acquisitions, mergers, an occasional unit spin-off, and frequent name changes. Corn Products Refining Co. and the Best Foods, Inc. merged in 1959, and later, in 1969, changed its name to CPC. International. In 1996, CPC. International had sales of $9.8 billion and it again split off Best Foods during 1997. Unilever acquired Best Foods in 2000 (6).

Edward A. Knaggs was chief chemist at Ninol Laboratories from 1945 to 1957. From 1957 to 1987, he held several research management positions at Stepan Co., rising through the ranks to vice president and general manager of the company's petroleum products division. Knaggs is the coauthor of 38 publications and 47 US and foreign patents. He can be contacted at +1 847-948-5918.

[FAST FACT]

How to have your doughnut and eat it too

How do you make a doughnut that not only has 40% less fat and 45% less saturated fat than the typical market average, but that consumers prefer?

Bakery supplies specialist CSM filed a patent for a pre-frying method where one side of the uncooked dough is sprayed for 2–4 seconds with hot oil at temperatures between 150–250° C. The doughnuts are then exposed to 20–600 seconds of infrared radiation and at least 1 second, but less than 20 seconds, of impingement with hot air at temperatures of at least 150°C.

According to Bakerysnacks.com, more than 71% of the 200 consumers who participated in sensory analysis testing found the reduced-fat version to be superior. The patent is available at http://tinyurl.com/ReducedFatDonut.

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E-sensing (cont. from page 288)

In addition to increasing the visual acceptability of products and packaging, e-eyes can be used to monitor product stability and shelf life, ensure batch-to-batch consistency, and assess surface homogeneity. "Cookie companies want to make sure that their chocolate chips are dispersed properly," says Shea. "Similarly, frozen food companies like their pizza materials to be distributed evenly."

Considering the improvements in e-sensing over the past few years, should professional food testers be worried about their job security? Not just yet, according to Aliani. "You need to be very careful when you're doing sensory work because there is not a direct comparison between the human nose and the electronic nose," he says. "All aroma compounds are volatile compounds, but not all volatile compounds are aroma compounds." In other words, the e-nose can detect compounds that the human nose doesn't recognize as smells. "Sometimes an e-nose will detect a lot of problems with a sample that a sensory panel doesn't," explains Aliani. "The e-nose is not doing exactly what our nose is doing. It's getting closer and closer, but I think we still have a long way to go before the e-nose replaces the human nose."

In the meantime, Aliani and many other researchers consider e-sensing to be complementary to sensory panels. "If you can correlate results from the e-nose and sensory panel, then and only then can you replace the human nose for that particular odor in that particular sample, and you don't need to do human panels any more," says Aliani.

Mifsud notes that e-sensing can alleviate the need for sensory panels during new product development. "It increases your capabilities to test different types of oil or packaging, different frying processes, or different flavors without being afraid that you will overwhelm the sensory panel," he says. E-sensors can narrow down new product options to the top two or three candidates, which can then be presented to the sensory panel for final selection.

Although e-sensors can tell you what odors and flavors emanate from your freshly baked cookie, and whether the chocolate chips and nuts are perfectly distributed, no machine can yet appreciate the hedonic aspects of a food. As such, e-sensors may never be able to fully replace sensory panels for determining which foods are the most enjoyable to eat. "The pleasure remains, thanks to God, in the courtyard of the human being," says Mifsud.

Laura Cassiday is a freelance science writer and editor based in Hudson, Colorado, USA. She has a Ph.D. in biochemistry from the Mayo Graduate School and can be contacted at lauracassiday@yahoo.com.

FIG. 2. *The Insent TS-5000Z taste sensor has eight sensors that selectively detect the five basic tastes: saltiness, sourness, umami, bitterness (three different types of bitterness), and sweetness. Credit: Kiyoshi Toko*

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Extracts & Distillates (cont. from page 311)

adducts with the cyclic acyloxonium free radical (CAFR) and its product MS ions, proving the presence of CAFR. Furthermore, the free radical mechanism was validated by the formation of 3-MCPD diesters through reacting DSG with a number of organic and inorganic chlorine sources including chlorine gas at 120°C and 240°C. The findings of this study might lead to the improvement of oil and food processing conditions to reduce the level of 3-MCPD diesters in foods and enhance food safety.

Spectroscopic determination of metals in palm oils from different stages of the technological process

Szydłowska-Czerniak, A., *et al.*, *J. Agric. Food Chem.* *61*:2276–2283, 2013.

Magnesium, calcium, copper, iron, and lead in palm oils (*Elaeis guineensis*) at various stages of the refining process were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. The mean concentrations of Mg, Ca, Cu, Fe, and Pb in the studied palm oils varied from 20.7 to 7090.1 μg kg^{-1}, from 193.9 to 8077.9 μg kg^{-1}, from 29.7 to 463.0 μg kg^{-1}, from 115.2 to 415.9 μg kg^{-1}, and from 1.7 to 16.0 μg kg^{-1}, respectively, which are below the Polish legal requirements. The comparable precisions for the proposed ICP-MS [RSD (relative standard deviation) = 0.81–5.99%] and standard graphite furnace atomic absorption spectrophotometry (GFAAS; RSD = 1.18–5.26%) methods demonstrate the benefit of the ICP-MS method in the routine analysis of metal ions in palm oils. There are significant positive correlations between Ca and Mg, between Ca and Cu, between Fe and Pb, between Cu and Fe, between Cu and Mg, and between Cu and Pb in palm oils determined by two analytical methods (r = 0.8798–0.9817, $p < 0.05$). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for discrimination of the quality of the analyzed palm oils based on main and trace metal contents determined by the proposed ICP-MS and the standard GFAAS methods. Two main groups were identified by HCA, whereas the classification and characterization of the studied palm oils within each of groups on the basis of metal ions amounts were obtained from PCA. The chemometric analyses demonstrated that crude palm oil had the highest level of the determined metals concentrations. Also, the analyzed metals in palm oils from different steps of the refining process were grouped using HCA to assess the effectiveness of technological processes for their removal.

More Extracts & Distillates can be found in the supplement to the digital edition of Inform. *Log in to read the May 2013 issue at www.aocs.org/login.*

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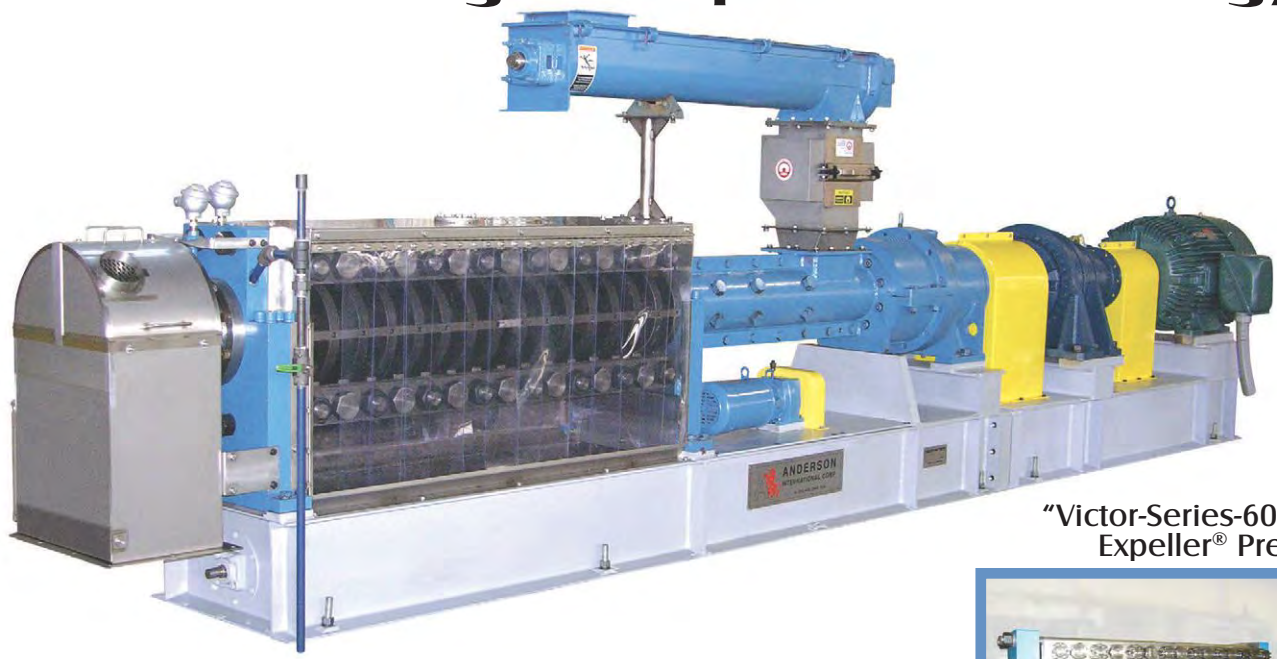

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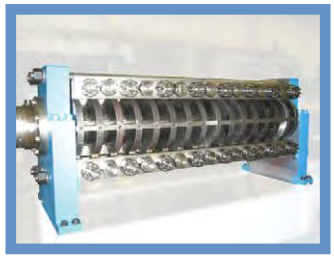

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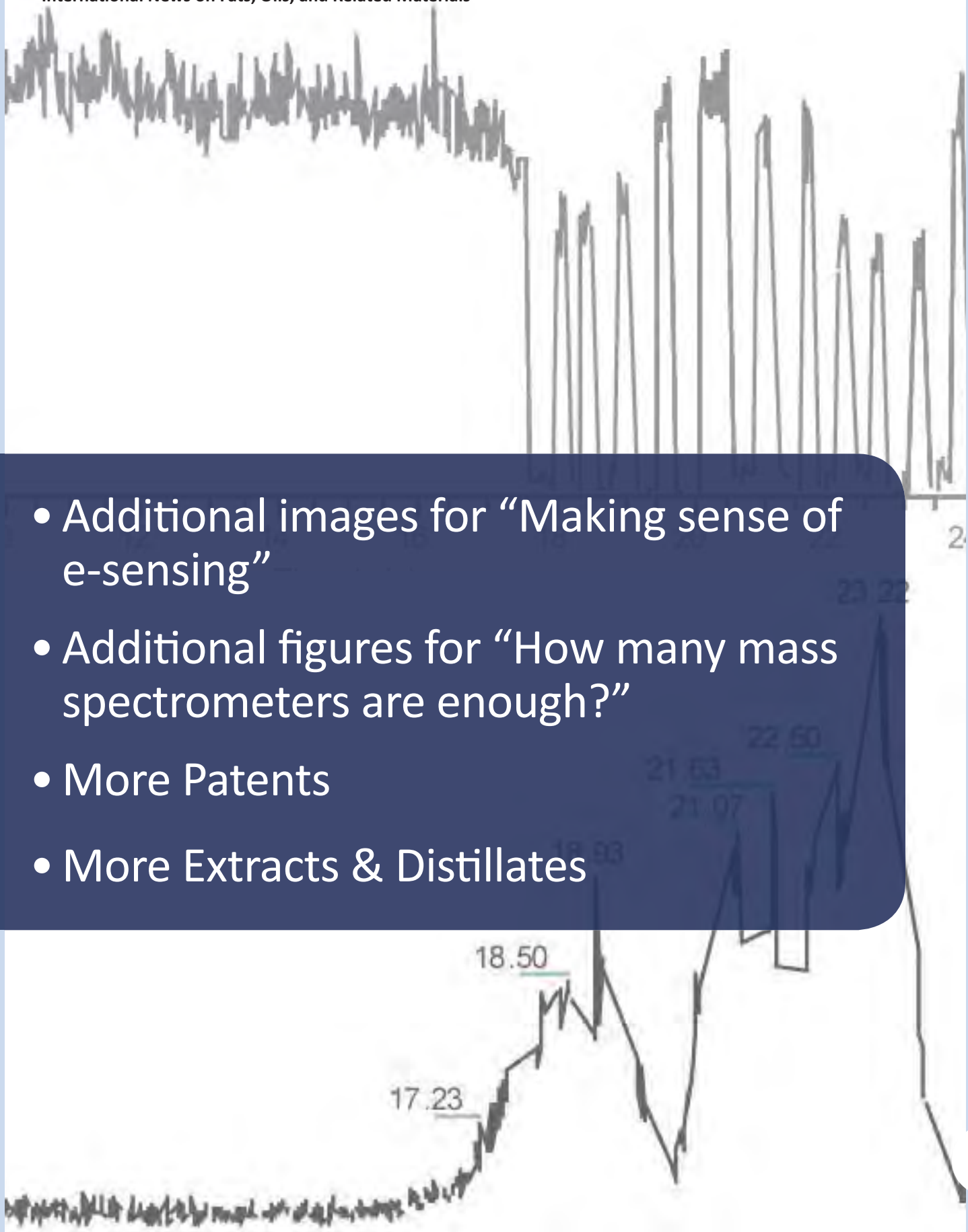
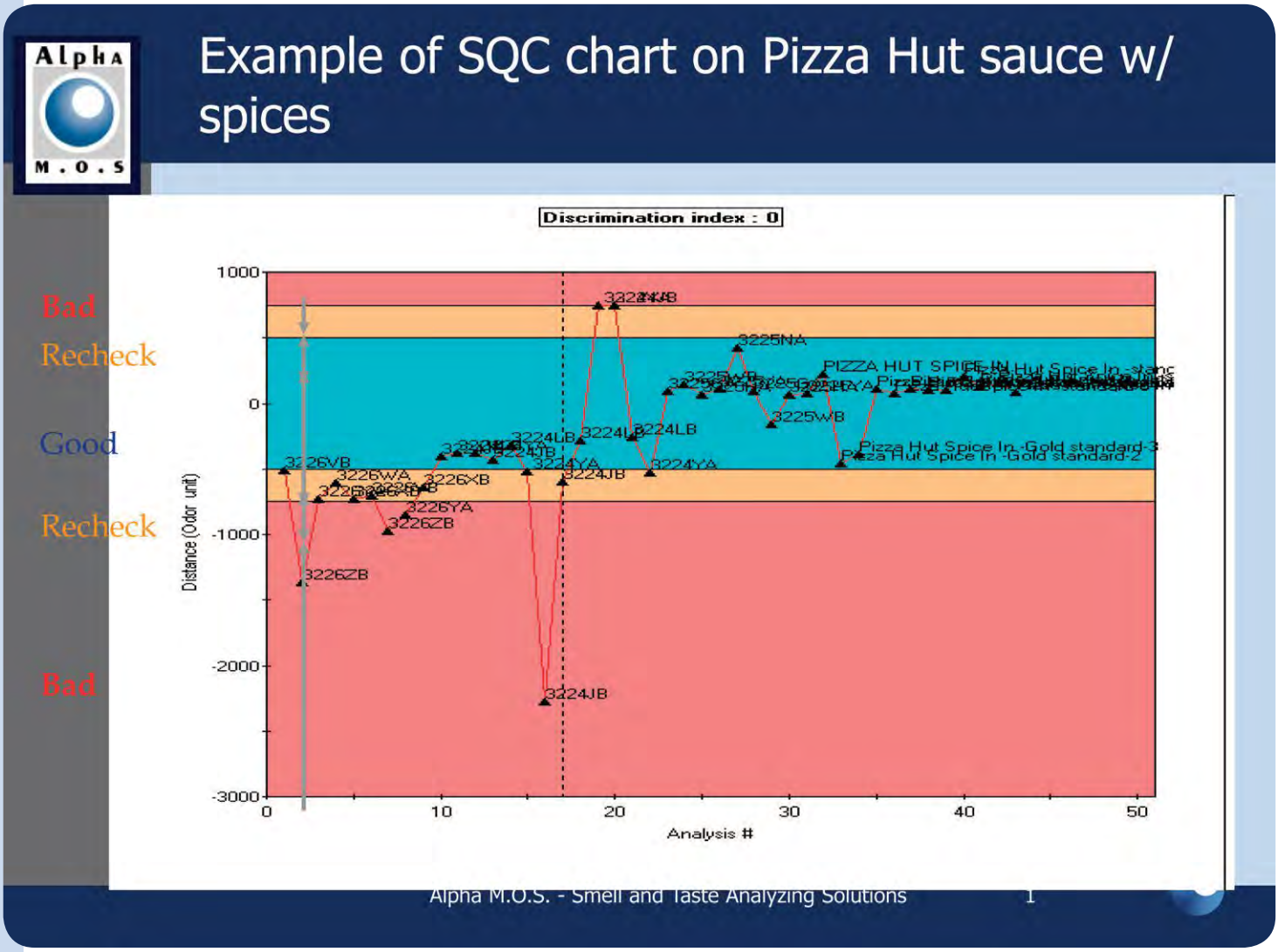


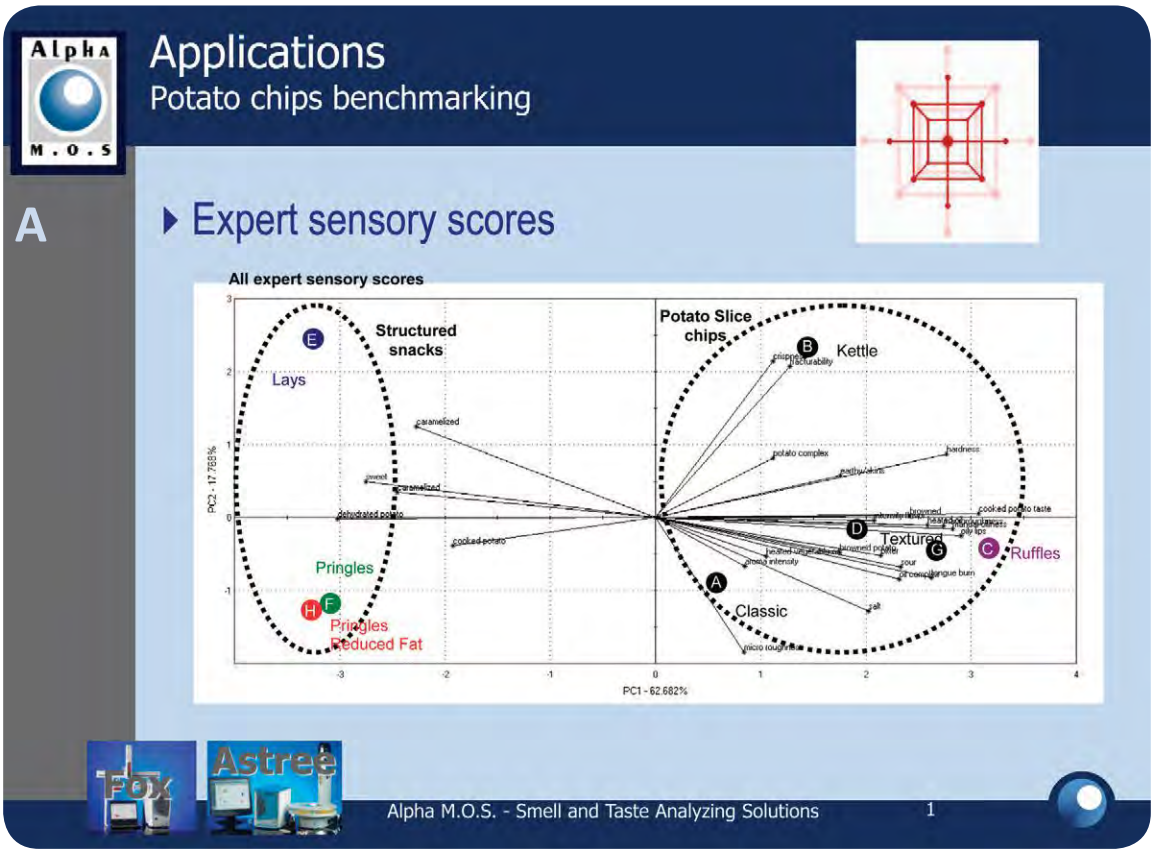
- Additional images for “Making sense of e-sensing”
- Additional figures for “How many mass spectrometers are enough?”
- More Patents
- More Extracts & Distillates

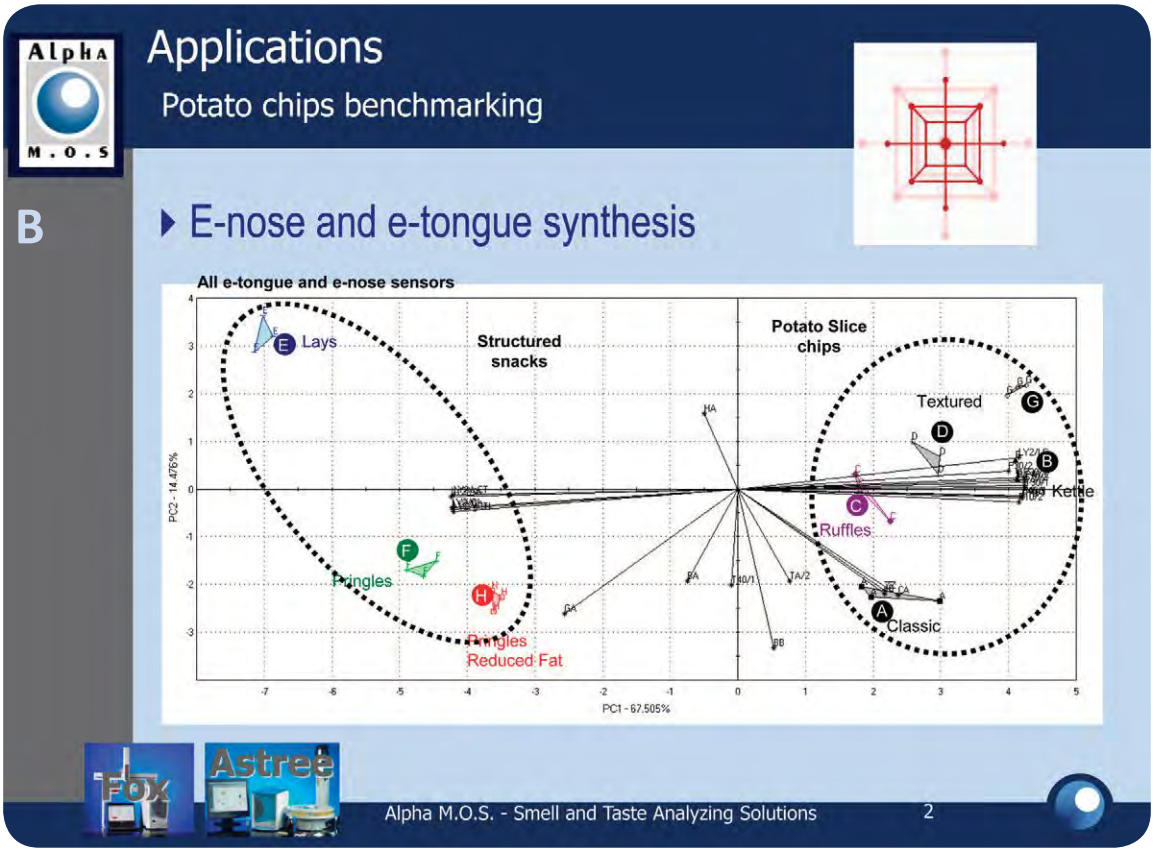
Making sense of e-sensing

These images supplement the article, “Making sense of e-sensing,” on page 284.



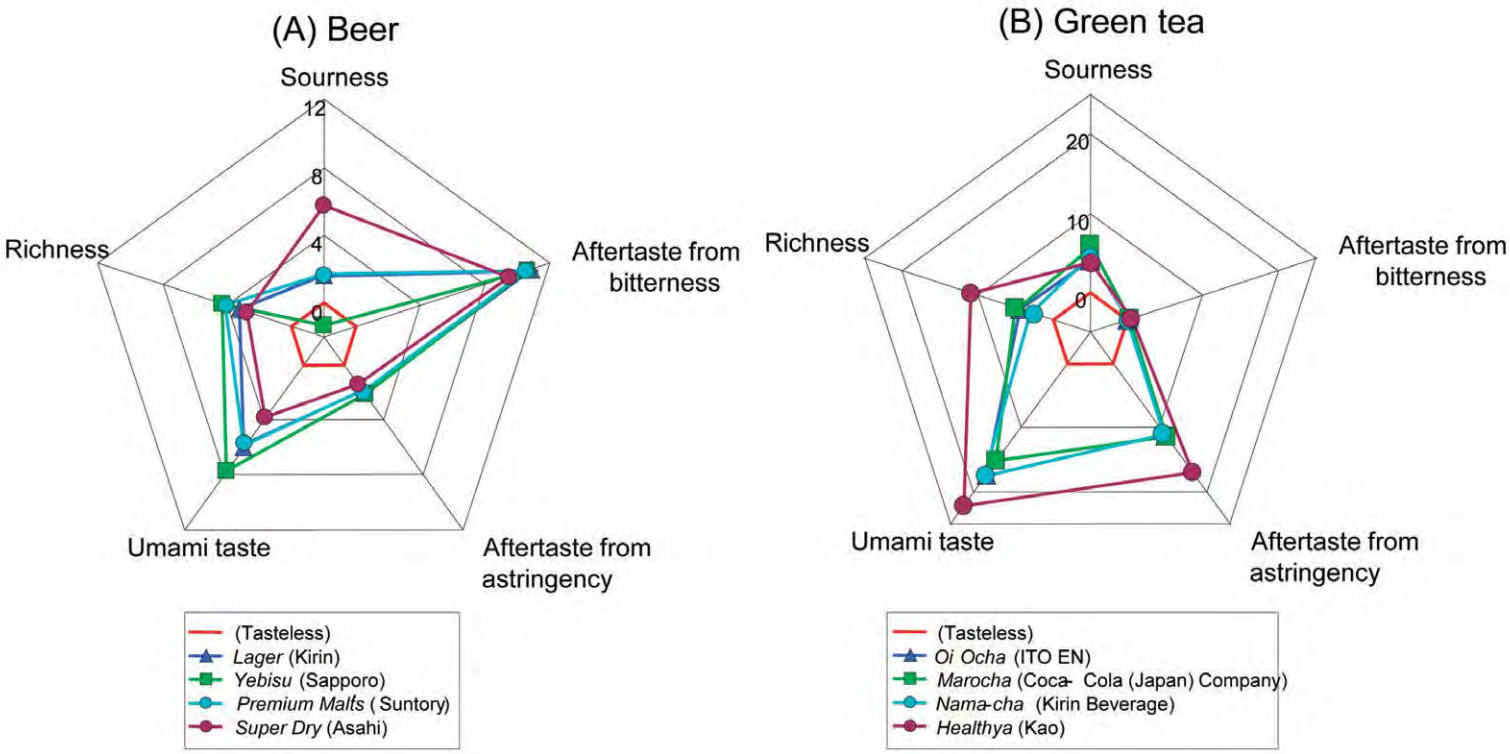
The Pizza Hut restaurant chain used Alpha MOS’s GEMINI e-nose to optimize the blend of spices and oil in their pizza sauce. The region in blue indicates the odor range for “good” samples, determined by training the e-nose with standards. Samples that fall within the orange zone should be rechecked, whereas the red zone indicates “bad” samples. Based on these results, Pizza Hut altered the production process to improve batch-to-batch consistency. SQC = statistical quality control. Credit: Jean-Christophe Mifsud





The sensory attributes of different brands of potato chips were compared using principal component (PC) analysis by an expert sensory panel (A) and the Alpha MOS FOX e-nose and ASTREE e-tongue (combined data; B). The sensory panel and artificial sensors discriminated the brands similarly. Credit: Jean-Christophe Mifsud

Radar Charts for Beer and Green Tea

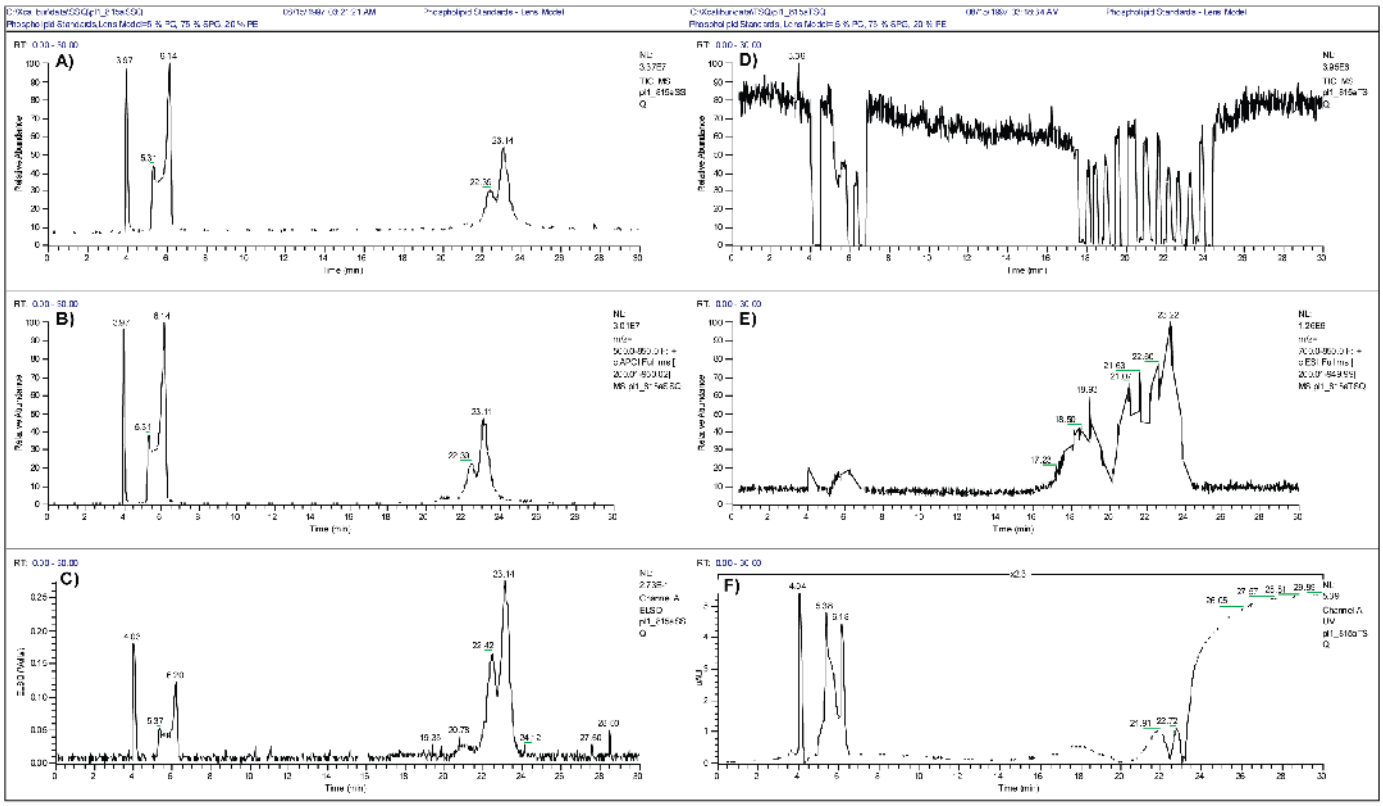


Reference solution : 30 mM KCl and 0.3 mM tartaric acid (regarded as tasteless sample set to zero)

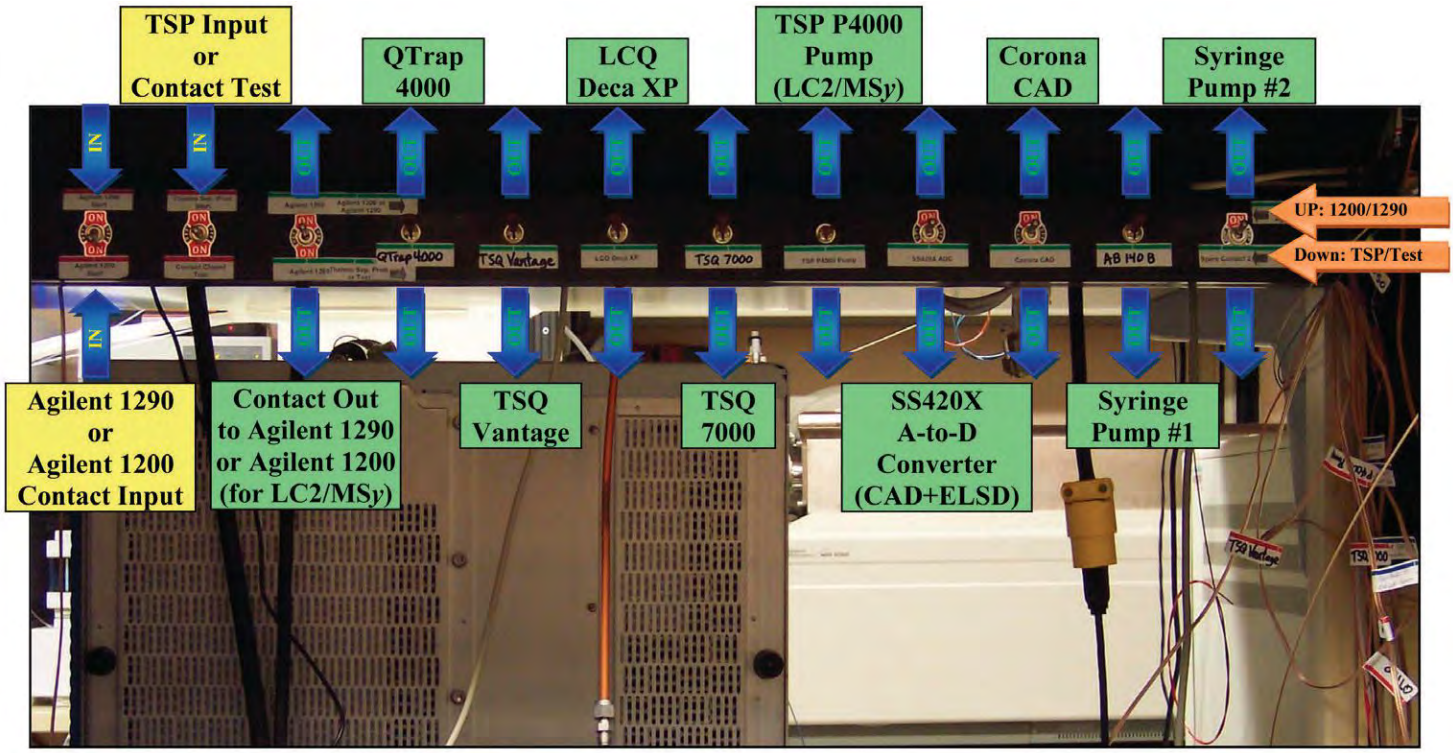
Radar charts illustrate the taste attributes of different brands of Japanese beer (A) and green tea (B), as determined by the Insent TS-5000Z taste sensor. Credit: Kobayashi, Y., M. Habara, H. Ikezazki, R. Chen, Y. Naito, and K. Toko, (2010) *Sensors* **10**: 3411–3443, (2010). doi:10.339/s100403411.

How many mass spectrometers are enough?

The following figures supplement the article, “How many mass spectrometers are enough?” on page 312.



First example of the analysis of phospholipids using atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) and electrospray ionization (ESI)-MS simultaneously, in parallel.(A) APCI-MS; (B) extracted ion chromatogram (EIC) of m/z 500–850; (C) evaporative light scattering detector (ELSD); (D) ESI-MS with auto-MS/MS; (E) EIC of ESI-MS full scans; (F) ultraviolet detection.



Contact closure distribution panel. LC autosampler contact closure inputs at left are distributed to any of 10 outputs to other LC systems, MS instruments, other detectors, and syringe pumps, etc. Abbreviations: LC, liquid chromatography; CAD, charged aerosol detector; for other abbreviations see Figure 1.

PATENTS

Process for preparing a bio-diesel

Wang, H., *et al.*, China Petroleum & Chemical Corp., Research Institute of Petroleum Processing; US8288573, October 16, 2012

The present invention provides a process for preparing a bio-diesel, comprising, in the presence of an alkaline metal compound, reacting an oil-fat with C_1–C_6 monohydric alcohol in a reactor at a reaction temperature of from 130 to 280°C and a reaction pressure of from 1 to 12 MPa, separating fatty acid esters from the reacted materials, so as to produce the bio-diesel, wherein said alkaline metal compound is present in an amount of 0.001–0.07 wt%, in terms of the metal thereof, relative to the weight of the oil-fat. The process provided in the present invention has the advantages of great throughput and high yield of the bio-diesel.

Process for preparing carboxylic acid derivatives

Boehmke, U., *et al.*, Evonik Rohmax Additives GmbH, US8288583, October 16, 2012

The present invention relates to processes for preparing carboxylic acid derivatives, comprising the reaction of at least one carboxylic acid and/or of a carboxylic acid derivative with at least one alcohol and/or an amine in the presence of a metal-containing catalyst, wherein, after the reaction has ended, the metal-containing catalyst is contacted with water and a superabsorbent, the contacting of the catalyst with the water leading to hydrolysis of the catalyst. The present invention further relates to the use of superabsorbents for removing a metal-containing catalyst from a mixture after hydrolysis of the catalyst.

Transesterified polyol having selectable and increased functionality and urethane material products formed using the polyol

Kurth, T.M., *et al.*, Tandem Polymers, Inc., US8333905, December 18, 2012

The present invention includes a polyol produced according to the process comprising reacting a multifunctional alcohol with a first multifunctional component to form a reaction product and reacting the reaction product with a vegetable oil to form a polyol. The present invention also relates to the material comprising the reaction product of an A-side and a B-side, wherein the A-side comprises an isocyanate and the B-side comprises the product formed by the process comprising the steps of reacting a multifunctional alcohol with a first multifunctional component to form a precursor polyol and then reacting the precursor polyol with a vegetable oil to form a vegetable-based polyol with selectable functionality.

Plasticiser alcohol and production improvement

Van Driessche, E., *et al.*, ExxonMobil Chemical Patents Inc., US8288595, October 16, 2012

Embodiments of the invention disclosed herein relate to a process for the production of a C_6–C_{15} alcohol mixture comprising the steps of: hydroformylating an olefin mixture comprising a branched C_5–C_{14} olefin to form a hydroformylation product comprising aldehydes and formate esters, whereby the hydroformylation product has a net cold sap number [saponification number?] from 15 to 38 mg KOH/g, and converting the aldehydes and formate esters to alcohols in a hydrogenation step comprising at least one first hydrogenation reactor comprising a fixed bed of a heterogeneous sulfided bimetallic catalyst.

Flexible polyurethane foams prepared using modified vegetable oil-based polyols

Herrington, R., and J. Malsam, Cargill, Inc., US8293808, October 23, 2012

A flexible polyurethane foam prepared by reacting, in the presence of a blowing agent, a polyisocyanate with an active hydrogen-containing composition that includes a modified vegetable oil-based polyol. The foams exhibit good load-bearing properties, relatively high sag factors, and/or good color retention upon exposure to light.

Carboxylic acid recovery and methods related thereto

Orjuela, A., *et al.*, Board of Trustees of Michigan State University, US8293935, October 23, 2012

A method of producing an alkyl ester of a carboxylic acid is provided, the method comprising: adding an alkanol and a mineral acid to a carboxylic acid salt to provide a carboxylic acid/alkanol solution and a precipitated mineral acid salt; separating the mineral acid salt from the carboxylic acid/alkanol solution; esterifying the carboxylic acid; and isolating an alkyl ester of the carboxylic acid.

Carbonate phase change materials

Kenar, J.C., United States of America as represented by the Secretary of Agriculture, US8354040, January 15, 2013

Phase change materials that include oleochemical carbonates absorb and release latent heat upon changing phases from solid to liquid (melting) or from liquid to solid (solidifying). The oleochemical carbonates are prepared from oleochemical alcohols

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derived from animal fats and vegetable oils or other bio-based substances. These oleochemical carbonates have melting temperatures with a relatively high heat of fusion and are non-corrosive. Oleochemical carbonates can be mixed together in various proportions to adjust melting/solidification temperature ranges as required by a particular application.

Process for using polyoxythylene sorbitan fatty acid ester

Wakita, K., *et al.,* NOF Corp., US8334397, December 18, 2012

A process for producing a polyoxyethylene sorbitan fatty acid ester in which the bitterness just after the production and the bitterness with the lapse of time is suppressed. The process of the invention includes reacting an ester (component A) of a fatty acid having 10 to 22 carbon atoms and a monohydric alcohol having 1 to 3 carbon atoms with at least either (component B) of sorbitol and sorbitan to form a sorbitan fatty acid ester and adding ethylene oxide to the sorbitan fatty acid ester, thereby producing a polyoxyethylene sorbitan fatty acid ester, which is characterized that the following steps (i) to (iv) are contained: (i) the component B is provided in the form of a 50 to 90% by weight aqueous solution and mixed with the component A and dehydration was performed until a water content in the system reaches 1.0% by weight or less based on the total weight of the components A and B; (ii) 1 to 10% by weight of a monohydric alcohol having 1 to 3 carbon atoms and 0.1 to 1.0% by weight of an alkali catalyst based on the total weight of the components A and B are added at 50–90°C; (iii) a transesterification reaction is performed under a nitrogen stream at a reaction temperature of 140–190°C to thereby obtain a sorbitan fatty acid ester; (iv) ethylene oxide is added thereto at a reaction temperature of 70–130°C.

Nut skin products

Chevaux, K.A., *et al.,* Mars, Inc., US8337917, December 25, 2012

The invention relates to products, including foods such as confectionary and pet foods, comprising nut skins. Exemplary nut skins are peanut and almond skins. The products may also contain cocoa polyphenol and/or L-arginine, and are useful for inducing vasodilation.

Method for increasing the content of docosahexaenoic acid in fat-containing materials or in fats and oils

Hayashi, M., *et al.,* University of Miyazaki, Nippon Suisan Kaisha, Ltd., US8349595, January 8, 2013

A process for producing highly unsaturated fatty acids comprising culturing a stramenopile capable of producing highly unsaturated fatty acids in a culture medium containing an inhibitor for fatty acid desaturases; fats-and-oils in which the content of highly unsaturated fatty acids, particularly docosahexaenoic acid produced by the relevant method, is increased. A method for enhancing the productivity of highly unsaturated fatty acids in stramenopiles, comprising culturing a stramenopile in a culture medium containing an inhibitor for fatty acid desaturases; stramenopiles having the enhanced productivity of highly unsaturated fatty acids, generated by the relevant method. Particularly, the process for producing highly unsaturated fatty acids, the method for enhancing the productivity of highly unsaturated fatty acids, and the stramenopile having the enhanced productivity of highly unsaturated fatty acids, wherein the stramenopile is a microorganism classified into Labyrinthulea.

Products containing highly unsaturated fatty acids for use by women during stages of preconception, pregnancy and lactation/post-partum

Van Elswyk, M., DSM IP Assets B.V., US8349895, January 8, 2013

A method and product for improving maternal and child health through nutrition. Omega-6 fatty acid and/or omega-3 fatty acid are provided to a woman and/or child prior to, during and/or after pregnancy to improve the health of the woman and her child. The ratios of the omega-6 and omega-3 fatty acids vary during various stages, e.g., pre-pregnancy, pregnancy and post-pregnancy. The omega-6 and omega-3 fatty acids can be in a variety of forms, such as at least one of highly purified algal oil comprising 70% by weight or more of the desired highly unsaturated fatty acids, triglyceride oil combined with phospholipid, phospholipid, protein and phospholipid combination, or dried marine microalgae.

Process for producing storage-stable seed crystals of cocoa butter and/or of chocolate masses

Fichtl, P., *et al.,* Uelzena eG, US8349383, January 8, 2013

The invention relates to a process for the production of seed crystals for chocolate products, which process comprises the following steps: Melting cocoa butter or chocolate masses, mixing the melted mass with a supercritical fluid, expanding the obtained solution in an expansion apparatus so that the formation of powder particles takes place, and separation of the powder particles from the gas. The seed crystals obtained in this manner have a high component of βV crystals and therefore offer advantages over mechanically produced seed crystals in the production of chocolate. The process can be carried out with high space-time yields and therefore offers economical advantages over traditional processes. Furthermore, the use of the produced seed crystals for producing chocolate products with advantageous organoleptic qualities is subject matter of the invention. ■

EXTRACTS & DISTILLATES

Profiling of metabolites in oil palm mesocarp at different stages of oil biosynthesis

Neoh, B.K., *et al., J. Agric. Food Chem. 61*:1920–1927, 2013.

Oil palm is one of the most productive oil-producing crops and can store up to 90% oil in its fruit mesocarp. However, the biosynthetic regulation and drivers of palm mesocarp development are still not well understood. Multiplatform metabolomics technology was used to profile palm metabolites during six critical stages of fruit development in order to better understand lipid biosynthesis. Significantly higher amino acid levels were observed in palm mesocarp preceding lipid biosynthesis. Nucleosides were found to be in high concentration during lipid biosynthesis, whereas levels of metabolites involved in the tricarboxylic acid cycle were more concentrated during early fruit development. Apart from insights into the regulation of metabolites during fruit development in oil palm, these results provide potentially useful metabolite yield markers and genes of interest for use in breeding programs.

Comprehensive profiling of carotenoids and fat-soluble vitamins in milk from different animal species by LC-DAD-MS/MS hyphenation

Gentili, A., *et al., J. Agric. Food Chem. 61*:1628–1639, 2013.

This paper describes a novel and efficient analytical method to define the profile of fat-soluble micronutrients in milk from different animal species. Overnight cold saponification was optimized as a simultaneous extraction procedure. Analytes were separated by nonaqueous reversed-phase (NARP) chromatography: carotenoids on a C30 column and fat-soluble vitamins on a tandem C18 column system. Besides 12 target analytes for which standards are available (all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, all-*trans*-β-carotene, all-*trans*-retinol, α-tocopherol, γ-tocopherol, δ-tocopherol, ergocalciferol, cholecalciferol, phylloquinone, and menaquinone-4), the DAD-MS [diode array detection-mass spectrometry] combined detection allowed the provisional identification of other carotenoids on the basis of the expected retention times, the absorbance spectra, and the mass spectrometric data. Retinol and α-tocopherol were the most abundant fat-soluble micronutrients and the only ones found in donkey's milk along with γ-tocopherol. Ewe's milk also proved to be a good source of vitamin K vitamers. Bovine milk showed a large variety of carotenoids that were absent in milk samples from other species with the only exception of all-*trans*-lutein and all-*trans*-zeaxanthin.

Adherence to the Mediterranean diet and body fat distribution in reproductive aged women

Boghossian, N.S., *et al., Eur. J. Clin. Nutr. 67*:289–294, 2013.

Adherence to the Mediterranean diet (MD), high in fruits, vegetables and monounsaturated fats, has been associated with lower body mass index. Associations with measured body fat, including regional adiposity, have not been previously investigated. We examined the associations between the alternate Mediterranean diet score (aMED), anthropometry, and measured adiposity by dual-energy X-ray absorptiometry (DXA). This study included 248 healthy females, aged 18–44 years, from the BioCycle Study. Each woman's aMED (range 0–9) was calculated from up to eight 24-h dietary recalls over 1–2 menstrual cycles (>97% had ≥7 recalls). Multiple linear regression was used to determine whether aMED and its specific components were associated with total and regional adiposity after adjusting for age, race, education, physical activity, and energy intake.

Participants had an average (standard deviation) aMED of 4.2 (1.7) and percent body fat of 29.5 (6.0)%. Significant inverse associations were found between aMED and all the examined adiposity measures except waist-to-hip ratio. Among the DXA measures, a 1-unit increment in aMED was associated with a 0.06 (95% confidence interval (CI): –0.09, –0.02) lower trunk-to-leg fat ratio (T/L), a measure of upper to lower body fat. In an analysis examining T/L as an outcome with the separate components of the aMED, T/L was lower with increased legume consumption (β = –0.280, 95% CI: –0.550, –0.010) but was higher with increased consumption of red and processed meat (β = 0.060, 95% CI: 0.002, 0.117).Adherence to the aMED was associated with lower total and regional adiposity, adding to the mounting evidence of the health benefits of the MD.

Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age

Much, D., *et al., Eur. J. Clin. Nutr. 67*:282–288, 2013.

Evidence is accumulating that the long-chain polyunsaturated fatty acids (LCPUFA) are associated with offspring growth and body composition. We investigated the relationship between LCPUFA in red blood cells (RBC) of pregnant

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women/breastfeeding mothers and umbilical cord RBC of their neonates with infant growth and body composition ≤1 year of age. In an open-label randomized, controlled trial, 208 healthy pregnant women received a dietary intervention [daily supplementation with 1200 mg n-3 LCPUFA and dietary counseling to reduce arachidonic acid (AA) intake] from the 15th week of gestation until 4 months of lactation or followed their habitual diet. Fatty acids of plasma phospholipids (PL) and RBC from maternal and cord blood were determined and associated with infant body weight, body mass index (BMI), lean body mass, and fat mass assessed by skinfold thickness measurements and ultrasonography. Dietary intervention significantly reduced the n-6/n-3 LCPUFA ratio in maternal and cord-blood plasma PL and RBC. Maternal RBC docosahexaenoic acid (DHA), n-3 LCPUFA, and n-6 LCPUFA at the 32nd week of gestation were positively related to birth weight. Maternal n-3 LCPUFA, n-6 LCPUFA, and AA were positively associated with birth length. Maternal RBC AA and n-6 LCPUFA were significantly negatively related to BMI and Ponderal Index at 1 year postpartum, but not to fat mass. Maternal DHA, AA, total n-3 LCPUFA, and n-6 LCPUFA might serve as prenatal growth factors, while n-6 LCPUFA also seem to regulate postnatal growth. The maternal n-6/n-3 LCPUFA ratio does not appear to have a role in adipose tissue development during early postnatal life. ■