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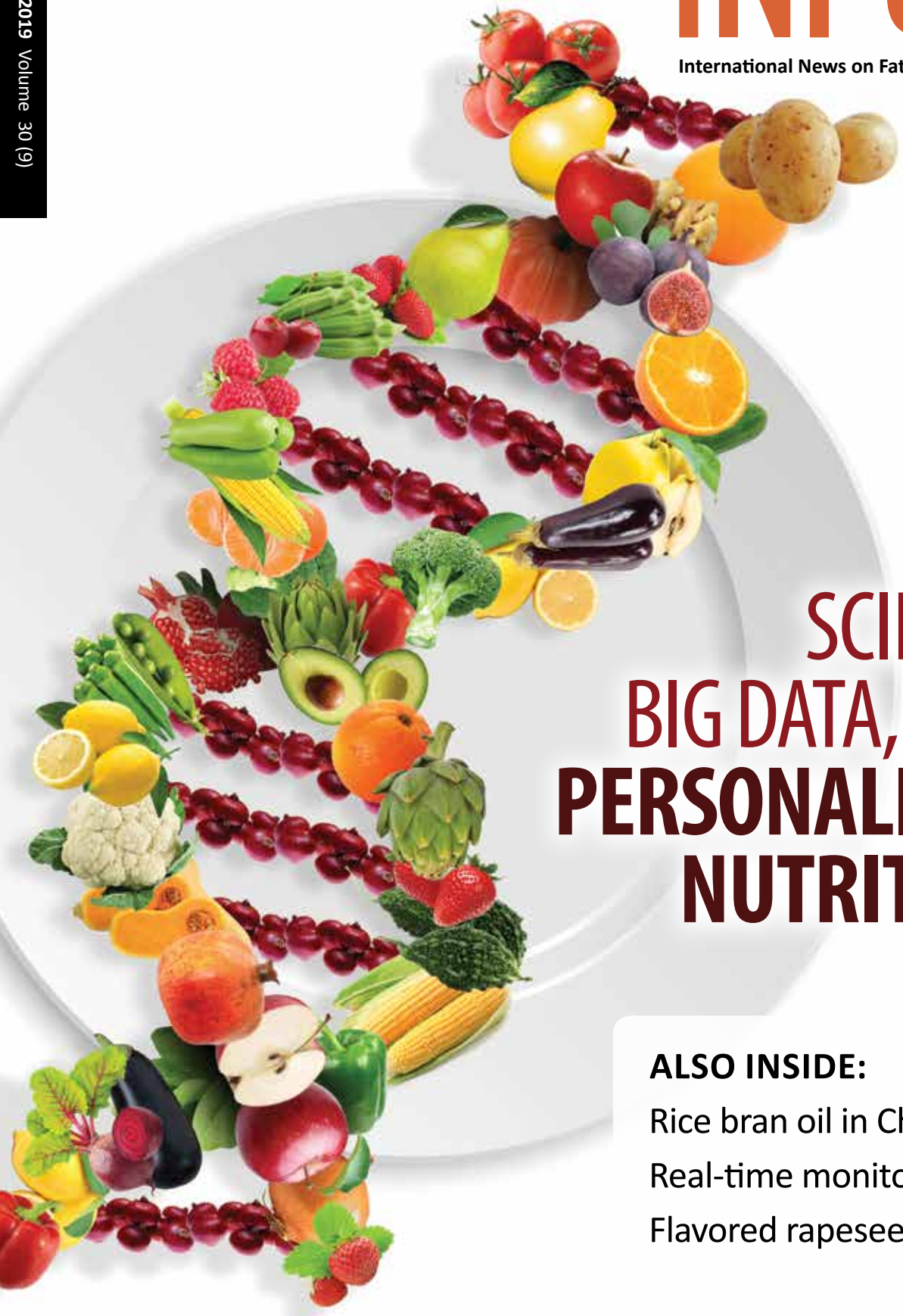

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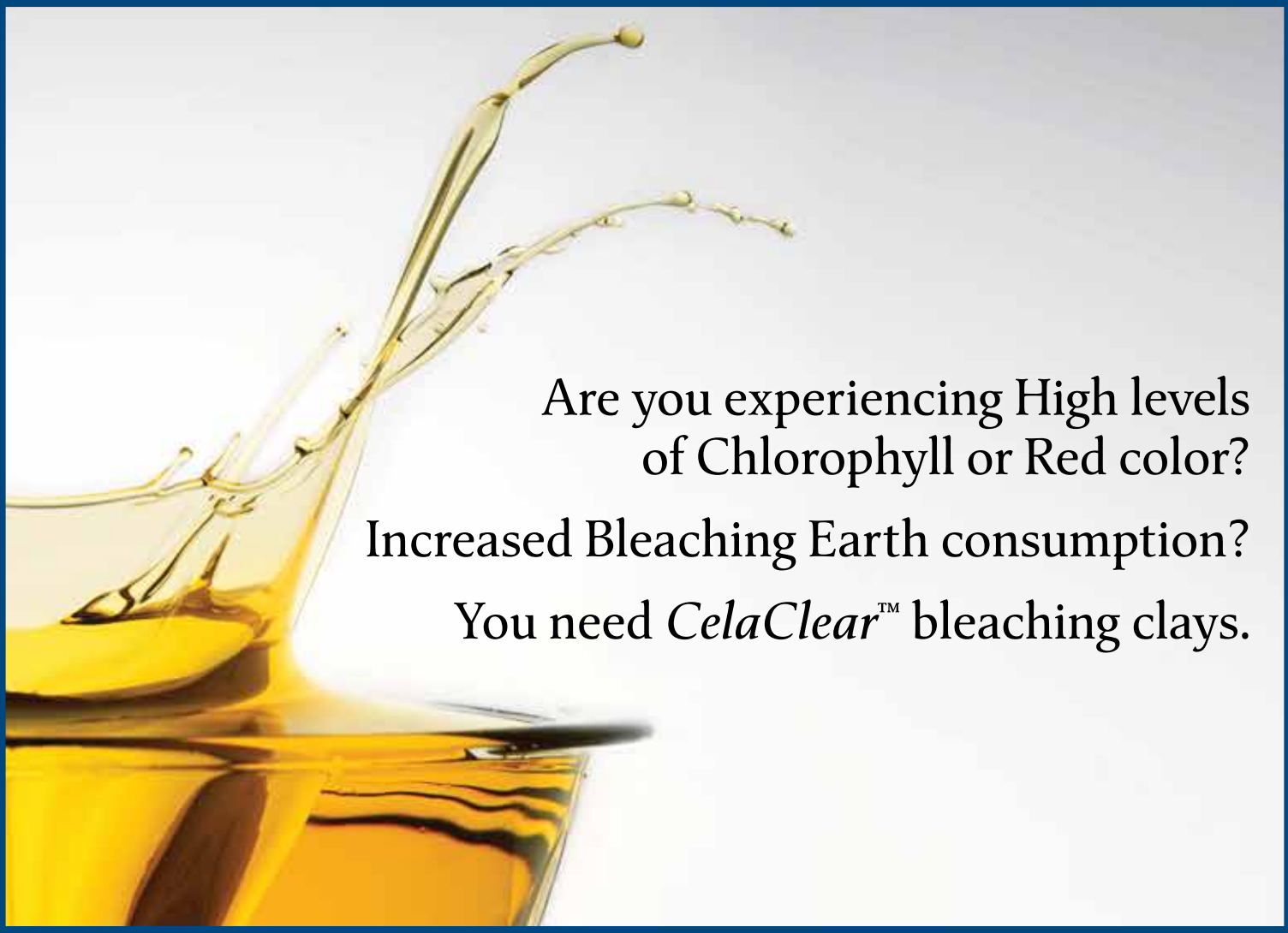

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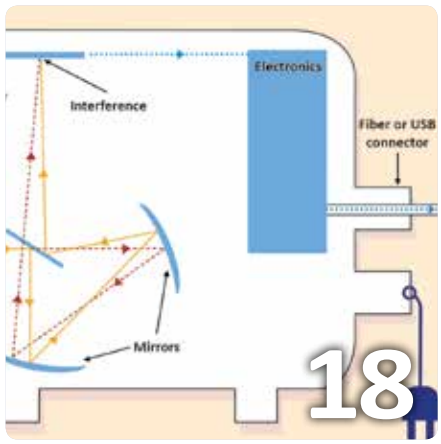

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Weaving together genetics, epigenetics, and the microbiome to optimize human nutrition

Rebecca Guenard

Like most of us, when Bryan eats potato chips his blood triglyceride levels spike, but when his twin brother eats the same chips his triglyceride levels are six times those of his genetic equivalent. When the Human Genome Project was launched in the early 1990s, scientists assumed that identifying our genes would reveal places in our DNA that can be manipulated for peak health. However, after four decades of research, the body's response to food has proven to be more complicated than they thought.

- **The human body holds the answers to its own optimal dietary needs, but can scientists decipher all the clues encompassing personalized nutrition to maximize our chances of a long life with the best possible health?**
- **Studies on identical twins diminish genetics' dominance in human health and highlight the importance of lifestyle choices.**
- **A better understanding of human populations and the function of the microbiome may help clarify the interaction between genetics and environment in human nutrition.**
- **Emerging evidence supports the notion that nutrition will be personalized in the future, since no one diet suits everybody.**

"We thought that genetics would explain everything," says Iwona Rudkowska, an associate professor at Laval University in Québec, Canada. "People thought, 'If we find out our genetics, then we will know exactly what we should eat. This is not the case."

Health is influenced by more than just our genes. Environmental factors, such as sleep and stress, can influence how genes function and affect our body's response to food. Now, more and more experts are stating that to improve public health, nutritional science needs to widen its scope to include details about individual responses to food.

Current dietary recommendations are based on the outcome of studies involving thousands of participants. "The outcome is the mean; how the average person responds," says Rudkowska. "But when you look at the data, some individuals respond completely differently than others."

The field of nutrition is now aiming for personalization. The goal is to deliver healthful eating guidelines tailored to the genetic, epigenetic, metabolomic, and microbiomic specifications of each individual. Large, long-term studies, like PREDICT and Food4Me are filling massive databases so computers can establish models that will forecast the best diet for an individual based on that person's unique parameters. Rudkowska says, "People are starting to realize that we are not all the same."

STRATIFICATION BEFORE PERSONALIZATION

Personalized nutrition stems from the notion of personalized medicine. The efficacy of certain cancer treatments, for example, are known to be influenced by a person's DNA. However, just as personalized medicine is not as turnkey as was once hoped, nutrition researchers are finding that genetic factors explain only a fraction of an individual's response to food.

For the past 10 years, Rudkowska has performed genome-wide association studies (GWAS) to evaluate if the effectiveness of omega-3 supplements are dependent on a genetic component. Her experiments typically involve hundreds of participants of Canadian decent whose plasma triglyceride levels are measured over several weeks while taking a supplement containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Rudkowska initially found four genes (IQCJ, NXPH1, PHF17, and MYB) that seemed to influence the body's response to omega-3s (https://doi.org/10.3390/ijms18020257). In other experiments, her group then identified 13 locations where the base pair of an individual whose triglycerides were lowered by omega-3s was different from individuals with no response (https://doi.org/10.1194/jlr.M045898). And, with finer genome mapping, her most recent experiments reveal 31 locations on the genome associated with the lowering of triglycerides (https://doi.org/10.1093/ajcn/nqy298).

Although her group has homed in on these potential genetic risk factors, Rudkowska says they cannot be used as personalized nutrition targets. "Even if we have a recommendation that a certain food will decrease cholesterol or LDL, that may not be true for everybody," she says. "The science is not there yet."

When glucose, insulin, or triglyceride blood levels are measured to study a person's response to a food or treatment, researchers find a wide variety of results. Some participants have a rapid and prolonged increase in blood sugar and insulin; others experience a spike in fat levels that lingers long after a meal. Either response could signal future problems like weight gain and diabetes or heart disease. However, when attempting to connect these blood results to a person's DNA, scientists have not been able to produce a strong correlation. Genetic factors account for less than 50% of a person's response to glucose. For insulin, it is less than 30%, and for triglycerides its under 20% (https://tinyurl.com/yyw3gbu8).

"We need to do more research before we can make exact recommendations on foods that one person would respond to better than another," says Rudkowska. For now, the best researchers can do with genetic data is to stratify people. Researchers who work in stratified nutrition consider dietary recommendations for specific groups, such as children, diabetics, or pregnant women, instead of individuals. "We know that individuals who have a higher risk of cardiovascular disease probably have higher triglycerides, so they might react better to omega-3s," she says. "We categorize it that way, but we are not at the point yet where we could use the genetic information, because there are so many other uncertainties."

Rudkowska is hoping that modeling will reduce some of the uncertainty and better predict responses. In parallel with her triglyceride experiments, she is studying the diverse effects that have been found between dairy consumption and blood glucose levels. Some researchers have identified effects of consuming dairy for individuals with type 2 diabetes, while others

have reported that dairy is detrimental to those with the disease (https://doi.org/10.1093/cdn/nzz083). Her group is collaborating with researchers who do bioinformatics to determine if they can find a biological source for the variability.

"We gathered data on microbiome, epigenetic, and metabolic factors. Then we used machine learning to develop an algorithm for predicting a person's response," Rudkowska says. With just one such factor, they can predict how dairy intake will affect the blood glucose level of a study participant with an accuracy of 65–70%. She says they hope to construct a better algorithm by incorporating multiple factors.

"I think we are moving away from just studying one gene and one food because it is too simplistic," says Rudkowska. "It is nice to think that we will react a certain way because of a genetic variation, but that is not what is happening. One person who has this variation might respond completely differently from someone else with the same variation." The results of ongoing studies with twins reiterate this fact. Nutritional studies now involve extensive data sets comparing every aspect of the lives of two people with the same genetics, yet different health outcomes.

TWINS AND EPIGENETICS

In 1992, Tim Spector, professor of genetic epidemiology at King's College in London, UK, started analyzing biological parameters of identical twins to see if he could uncover a genetic reason for osteoporosis and other rheumatologic diseases (www.twinsuk.ac.uk). He started out with a cohort of a few hundred participants that bloomed into a registry of 12,000 twins in the UK. Spector and his team collected blood, urine, and tissue from the twins, who were mostly middle-aged women, with equal numbers of identical and fraternal twins.

Over the decades, the program has provided a trove of genetic information on healthful aging and complex diseases. The data stored in the TwinsUK database is available to the scientific community, and it has prompted extensive research on everything from cardiovascular and metabolic disorders to behavioral and socio-economic characteristics of the population. This is due to the database being a motherload of systems biology resources: genome-wide scans of single nucleotide variants, RNA sequencing, metabolic profiles, gut flora microbiomics, epigenetic markers, and gene expression arrays, to name a few.

Some of the most intriguing results to arise from TwinsUK, reveal how lifestyle has a direct effect on gene expression. Genes are regulated by molecules that piggyback onto DNA and determine if the gene participates in the functions of the human body. This system is known as epigenetics.

Spector and his team reported in a 2005 paper that identical twins are epigenetically indistinguishable during their early years of life. However, when they compared the genetic information of 3-year-old identical twins to 50-year-old identical twins, they found that the epigenetics of the older twins were notably different (https://doi.org/10.1073/pnas.0500398102). Although each set of twins were born with the exact same DNA, the expression of their genes diverged as they aged and

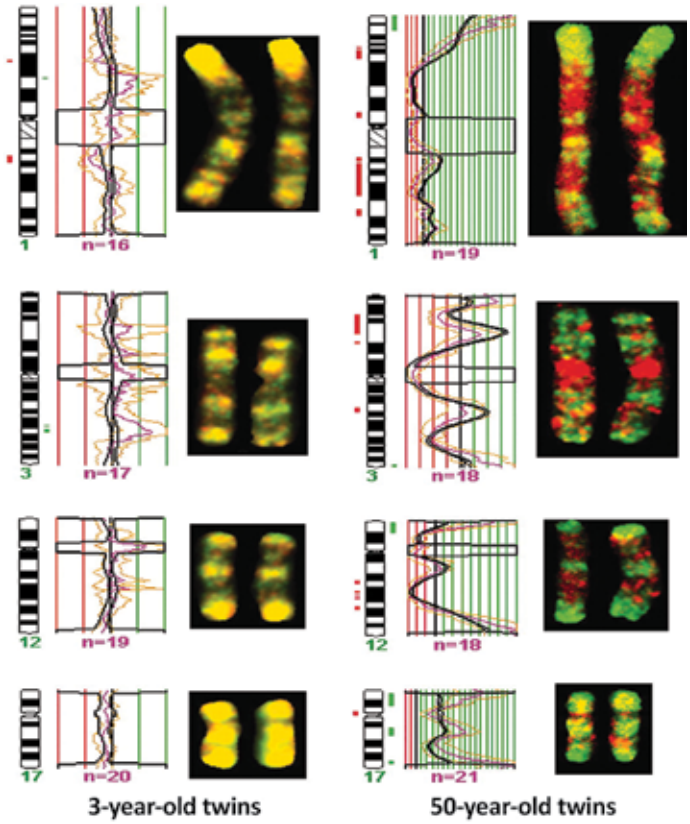


FIG. 1. Chromosomal regions of 3- and 50-year-old identical twins showing the epigenetic change of DNA methylation. Green and red areas show changes on the chromosome where methylation occurred, whereas the 3-year-old twins have similar methylation distribution indicated by their consistent yellow color.
Source: Fraga, *et al.*, *PNAS 102*: 30, 2005.

experienced different things. Since these early, intriguing studies Spector's team has pursued an ambitious goal of explaining the human's vast variability in response to diet (Fig. 1).

In June of 2019, the PREDICT 1 study published its initial findings and launched the second phase of the experiment, PREDICT 2 (https://predict.study/). Self-reported nutritional studies contain the inherent flaw that humans are notoriously bad at remembering what they ate. The PREDICT study avoided this by giving each participate a full clinical work-up prior to starting. Everyone then ate standardized meals in the clinic on the first day. Their blood was drawn 10 times and measured for sugar, insulin, fat, and inflammation biomarkers, among others. For the following two weeks, participants ate their own meals along with standardized foods. A wearable monitor recorded their blood sugar level every 15 minutes, and they took regular blood samples. Data collection on the participants was quite comprehensive, including monitored sleep and activity patterns, as well as regular stool samples.

The study concluded that the genetic contribution to the body's metabolic response is minor. There is little relevance in amount of fat or carbohydrates in a participant's food either. According to the PREDICT 1 report, the most important determinants of a healthy metabolic response were sleep, stress, exercise, and the gut microbiome.

GUT CHECK

As scientists dig through the ecosystem of the human intestinal tract attempting to parse out the role of billions of microbes, reports on new insights seem never-ending. Most recently, a team at Stanford University School of Medicine in Palo Alto, California, USA, discovered that the microbiome produces thousands of small proteins that were previously unknown and whose purpose remains to be understood (https://tinyurl.com/yyzcb4pz). As scientists unearth a constant flux of information about the microscopic landscape within the gut, each new find lends credence to the growing acknowledgement that nutritional choices affect the bacterial population ensconced there.

"The microbiota have many established roles in promoting host health," says Lauren Rajakovich, postdoc in the laboratory of Emily Balskus at Harvard University in Boston, Massachusetts, USA. She says there is still many unanswered questions, but mouse models designed with no microbiome relay the importance of these intestinal colonies.

"These model systems often have an underdeveloped immune system, so they are susceptible to different pathogens," she says. "They need a lot of supplementation in their diets." Without a microbiome, such mice cannot maintain good health. Scientists have established that the microbiome is needed for the biosynthesis of specific molecules, like vitamins, that are not formed anywhere else in the host's digestive system. Through these mouse models, researchers have determined that gut bacteria provide a last-ditch source of energy and nutrients as food waste exits the body.

"Food components that the human body is not able to digest end up in our large intestine, and the microbes that live there are able to break down these complex polysaccharides into smaller units that are reabsorbed by the host," Rajakovich says. She adds that the carbohydrates are reduced to simple sugars that the microbes convert into short-chain fatty acids used as an energy source by epithelial cells in the large intestine (Fig. 2).

The Balskus lab deciphers common metabolic pathways within the microbiome by concentrating on small molecule chemistry. Gut bacteria metabolize the contents of the intestines for their own nutritional and energy needs. Sometimes the small molecules they produce benefit the host by, for example, producing molecules that regulate immune responses, but sometimes gut bacteria dispel molecules that cause disease.

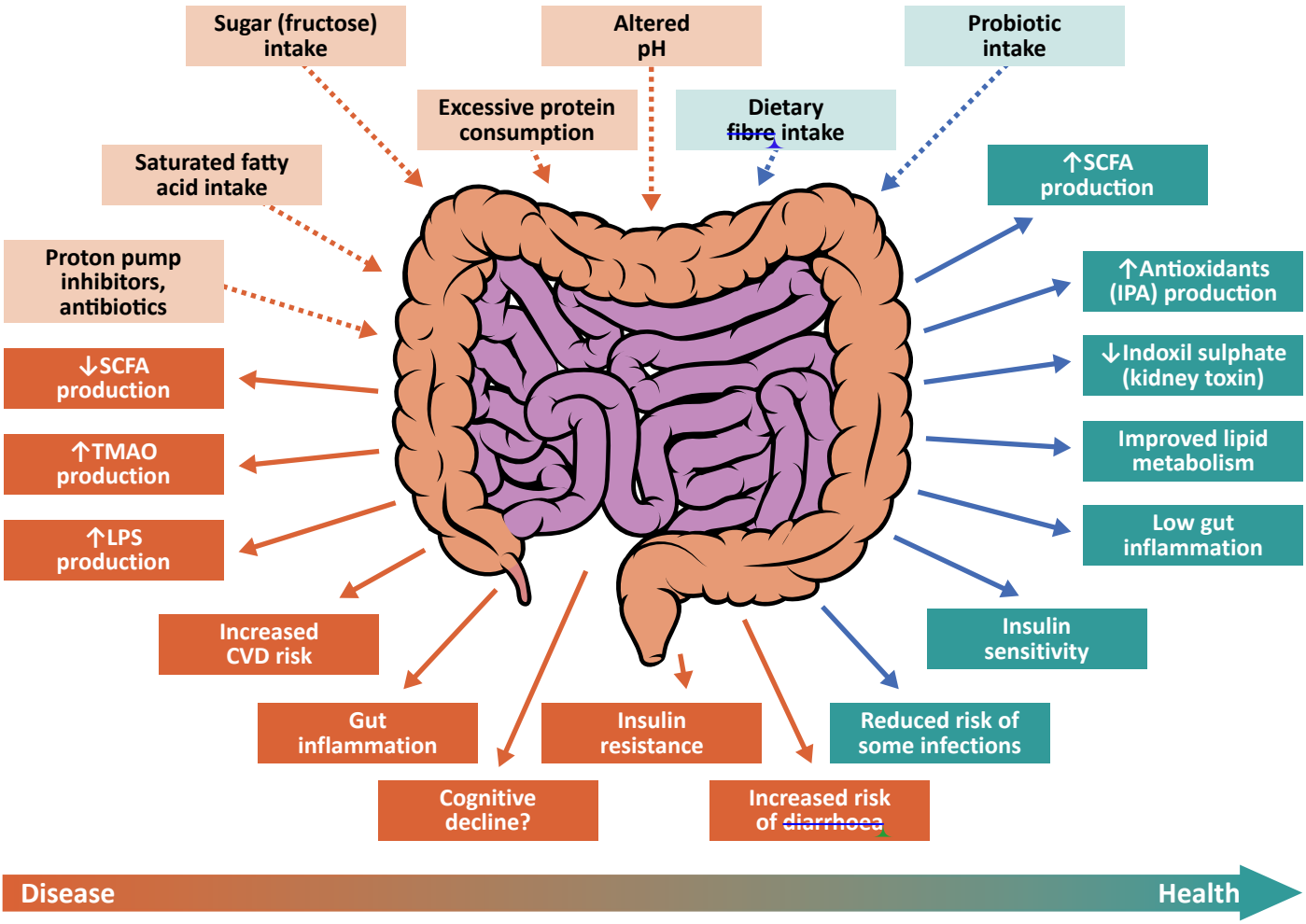
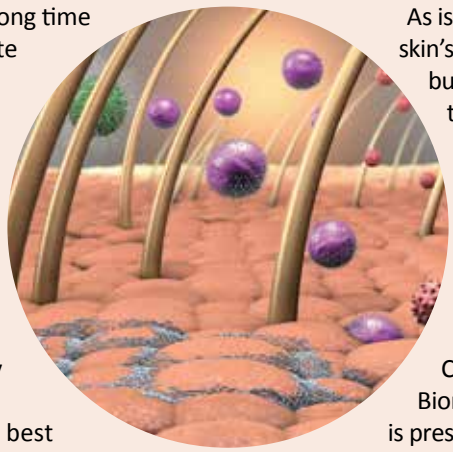


FIG. 2. Schematic representation of the role of the gut microbiota in health and disease giving some examples of inputs and outputs. CVD=cardiovascular disease; IPA=indolepropionic acid; LPS=lipopolysaccharide; SCFA=short chain fatty acid; TMAO=trimethylamine N-oxide. Source: Valdes, *et al.*, *BMJ: 361*: k2179, 2018.

Feeding the skin microbiome



Dermatologists have understood for a long time that bacteria on the skin's surface irritate hair follicles that cause acne, which is routinely combatted with prescription antibiotics. Just as antibiotics in the human gut eliminate good bacteria, most acne treatments do the same thing in skin.

The latest research suggests that instead of annihilating bacterial colonies, skin treatments should foster an environment for growth. Beauty companies are now taking stock of the skin's microorganisms to evaluate how best to promote skin health and treat a range of conditions.

Billions of microorganisms, including bacteria, fungi, mites, and viruses, can stake claim to a single square centimeter of skin, but bacteria are considered the most useful. They are present on the surface and in deeper layers, indirectly protecting the skin against pathogens by competing for resources, and directly by producing antimicrobial compounds—some of which release anti-inflammatory compounds. Not all bacteria share the same list of attributes, however, so cosmetic companies are trying to differentiate and promote the superstars.

As is the case with the gut, the science of the skin's microbiome is still being established, but beauty companies are already using the concept in new product lines, especially for the treatment of acne. Earlier this year, *Stat News* reported on two start-ups that are betting on the benefits of the microbiome (https://tinyurl.com/y4l24tfe). Both companies were launched within a Johnson & Johnson tech incubator located in San Francisco, California. One of the start-ups, Naked Biome, sequenced a strand of bacteria that is present on people with healthy skin and developed a cleansing pad soaked in the specific microbe. They are currently gathering data on the product for a future FDA filing. The other start-up, Ellis Day, is creating a serum containing three bacteria-killing viruses that they plan to market as an over-the-counter product.

With time, beauty companies will know if consumer success can be gleaned from a skincare product made from live viruses and bacteria. But companies like Johnson and &Johnson and Henkel have identified the microbes populating our skin as beauty industry game-changers and will continue to put scientific resources into determining how to use them to their advantage.

"Our lab is really excited about exploring the production of trimethylamine by the gut microbiome, because it has not only been associated primarily with cardiovascular disease, but also with chronic kidney disease, nonalcoholic fatty liver disease, and systemic things, like obesity and diabetes," Rajakovich says. The small molecule trimethylamine (TMA) is not made anywhere in the body except in the gut. Intestinal microbes convert choline from a variety of foods, such as, red meat, eggs, soy, or dairy, into TMA that is converted to trimethylamine oxide (TMAO) in the liver. TMAO is linked to plaque buildup and cardiovascular disease. The Balskus research group is exploring whether in the future a therapeutic could be administered to patients to reroute this metabolic pathway away from disease.

Many microbiome studies focus on bacterial metabolic pathways, with an eye toward treatment. For example, prebiotics have become a popular way to ramp up beneficial bacteria. A fiber-rich diet is known to promote an optimal ratio for glucose-regulating bacteria.

Details of various aspects of the microbiome are being discovered but designing personalized nutrition will require largescale studies that incorporate environmental as well as dietary factors. "We often focus in on a particular bacterium and sometimes we do forget that they are part of this much larger and complex community that is all interconnected," says Rajakovich. She adds that the study of human gut microbiota would really benefit if ecologists and population geneticists worked together to figure out such complex systems, while chemists and biochemists continue to explore the metabolism.

THE POCKET NUTRITIONIST

The implementation of high-throughput tools for genomics, microbiomics, and metabolomics, together with the computational capabilities of artificial intelligence (AI), have led to the opportunity to create a customized nutritional plan for anyone. Just as 23andMe and AncestryDNA offer to give customers a glimpse into their heritage, a similar industry is forming around personalized nutrition.

The Santa Clara, California-based microbiome analysis company, Viome, launched in 2019 with the ambition to "take the guesswork out of eating right with a science-based, at-home microbiome test," (https://www.viome.com/). Viome analyzes the genes expressed by the microbiome, labels them good or bad, and advises customers about foods that will improve their health. Spector has launched a product based on his TwinsUK research. His app for your phone, ZOE, describes the best diet for a user's metabolism (https://joinzoe.com/).

The interesting thing about both products is that customer information will contribute to a database that will use AI to better predict personalized nutrition for future users. The more people purchase the products, the more data gets added to the AI algorithm and, presumably, the better it will be at guessing a consumer's specific dietary needs. Such big data establishes a predictive model that can be used to evaluate an individual's response to a nutritional intervention, while also providing nutrition experts with an understanding of how diet influences health and disease.

"One caveat with this type of product is that what we understand about the gut microbiome is that it is very flexible and temporal," Rajakovich. "The difference between that and 23andMe is that your genetics is never changing."

Rudkowska says researchers still have a lot to resolve concerning the microbiome, but these big data studies are the future of nutrition. She says the technology to analyze all the body's biological parameters is now available; the data is easy to acquire. The challenge is how to interpret all the information researchers collect.

"These studies are complicated," she says. "But the human body is complicated."

Rebecca Guenard is the associate editor of Inform *at AOCS. She can be contacted at rebecca.guenard@aocs.org.*

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Rice bran oil in China: opportunities and challenges

Yuanrong Jiang, Yong Wang, Lvrui Liu, and Junmei Liang

- Rice bran oil (RBO) is considered to be a healthful oil, as it contains large quantities of micronutrients and balanced fatty acids, such as triacylglycerol. China produces more rice than any country in the world, and the health benefits and abundance of this raw material have increased the popularity of rice bran there.
- The high free fatty acids (FFA) content of crude rice bran oil and over-refining are major problems in RBO processing. A comprehensive set of solutions (in-time stabilization technology, enzymatic refining, mild processing, etc.) have been developed to address these problems.
- An innovative model of the rice industrial chain has also been established to increase the overall utilization and sustainability of the rice-producing process. The development of underused by-products, such as lecithin and wax, will lead to more value-added products.

RBO PRODUCTION IN CHINA

China ranks first in the production of rice globally. In 2018, there were 210 million metric tons (MMT) of rice produced in China, which accounted for 28% of the world's total production (Fig. 1)

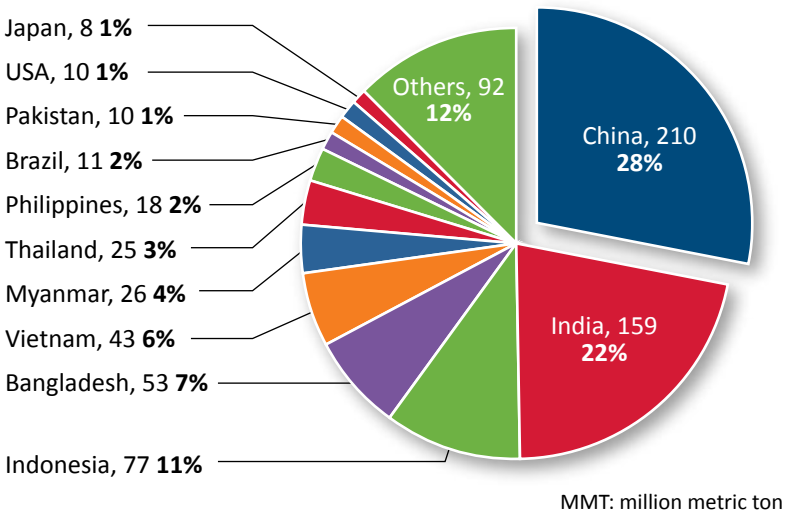


FIG. 1. Composition of global rice production. The total rice production was 750 million metric tons (MMT) in 2018. Source: USDA, 2018

Rice must go through dozens of processing procedures as it makes its way from farm to table, during which about 30% of the total weight is taken away. Rice bran is one of the by-products. About 15–18% of rice bran is fat, and abundant micro-nutrients are left in it. Although China produces more rice than any other country, the percentage of rice bran used to produce RBO is much lower in China than it is in Japan or India (Table 1).

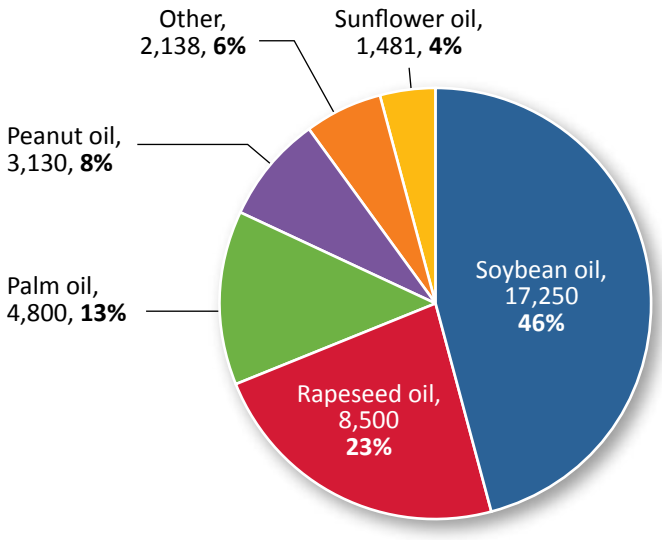
Meanwhile, domestic consumption of edible oil in China outstrips production. About two-thirds of the edible oils in the market is imported. Figure 2 shows the domestic oil consumption trend in 2017. It is difficult to increase domestic yields of conventional oils due to limited

TABLE 1. Rice bran utilization rate for RBO in various countries. Source: FAO, 2018

Country	RBO Potential /KMT	RBO Production /KMT	Utilization /%
China	2300	440	20
India	1620	960	59
Japan	105	97	92

KT: kiloton

FIG. 2. Composition of domestic oil supply in China/KMT.
Source: USDA, 2018



arable farmland. However, since RBO is a by-product of existing rice production and does not require additional farmland, RBO production has attracted government support.

The government's National Five-Year Plan is the uppermost development guideline for the Chinese economy. It states that "by year of 2020, the utilization rate for RBO will achieve 50%." Previous standards for RBO were set to be exceedingly strict due to a lack of recognition. The national standard for crude RBO has since been adjusted to be more practical, in the hopes that this will encourage more companies to participate in RBO production. RBO production has developed gradually over the past 10 years. Some producers have made efforts to reduce waste and improve utilization throughout the whole rice industry chain. Wilmar-International is one of the leading agriculture companies in China, with businesses that produce edible oil, rice, and flour. A comprehensive, waste-free rice industry chain has been established by the company (Fig. 3, page 14). Rice bran for oil extraction is a key step in that chain.

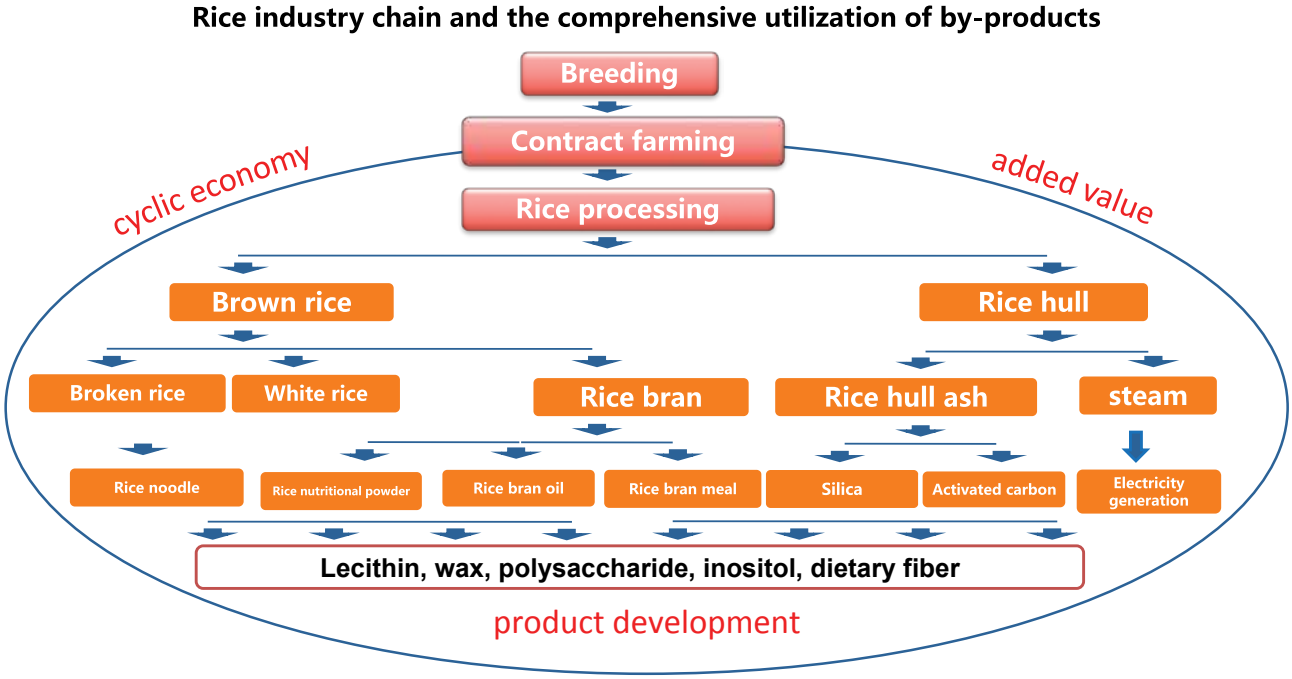


FIG.3 Wilmar Industry Chain. Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

NUTRITIONAL VALUE

Crude RBO is composed of triacylglycerol (68%–71%), diacylglycerol (2%–3%), monoacylglycerol (5%–6%), glycolipids (5%–7%), phospholipids (0.5–0.8%), free fatty acids (2%–3%), wax (2%–3%), and unsaponifiable lipids (4%).

Table 2 shows the fatty-acid composition of RBO and five other commonly used oils. Refined RBO contains 36% of linoleic acid and 43% of oleic acid. This balanced fatty-acid profile may provide a good solution to reducing overconsumption of omega-6 or omega-9 fatty acids.

RBO also contains high levels of micronutrients like γ-oryzanol, phytosterols, and vitamin E. Figure 4 compares RBO's minor components with those of seven other oils. These minor components enhance the nutritional value of RBO. γ-oryzanol is the ester of ferulic acid and tocopherols. It has been shown to have antihypertensive and hyperglycemic effects, and is reported to have a positive effect on lowering the risk of cardiovascular diseases by competing with low-density lipoprotein (LDL) cholesterols (Chow *et al.*; Shakib *et al.*; Justo *et al.*).

PROCESSING CHALLENGES

Rancidity is a core problem in RBO processing. Fresh bran must be stabilized, because the fat in rice bran is hydrolyzed quickly by the catalysis of the lipase after polishing. The free fatty acids can easily rise to 10–12% within 24 hours, and the oil starts to have a funky smell. Rice bran stabilization is the most important step in ensuring RBO quality, and highly rancid rice bran leads to serious production losses and higher energy costs. Yet, the small-scale rice processing factories dispersed throughout China are seldom equipped with stabilization facilities, which makes it extremely difficult to produce quality certified RBO.

Fortunately, some leading companies have started to develop localized stabilization techniques. Several effective solutions for rice bran stabilization have been used in recent years. Extrusion is a widely used method for lipase inactivation. The acid value (AV) of stabilized rice bran can be maintained at less than 6 mg KOH/g for 8 months after extrusion. In recent years, more and more companies have set up small extruders (10 tons/day) in their small-scale mills so that rice bran can be stabilized on site. The stabilized rice bran is then

TABLE 2. Fatty-acid composition of various oils. Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

	Saturated fatty acid	Unsaturated fatty acid			
		Oleic acid (ω 9)	Essential fatty acid		SFA:MUFA:PUFA
			Linoleic acid (ω 6)	Linolenic acid (ω 3)	
RBO	19	43	36	1	0.4:1:0.9
Canola oil	6	64	20	9	0.1 :1:0.5
Soybean oil	14	22	55	8	0.6:1:2.9
Olive oil	15	75	8	1	0.2:1:0.1
Sun flower oil	12	25	62	0.5	0.5: 1:2.5

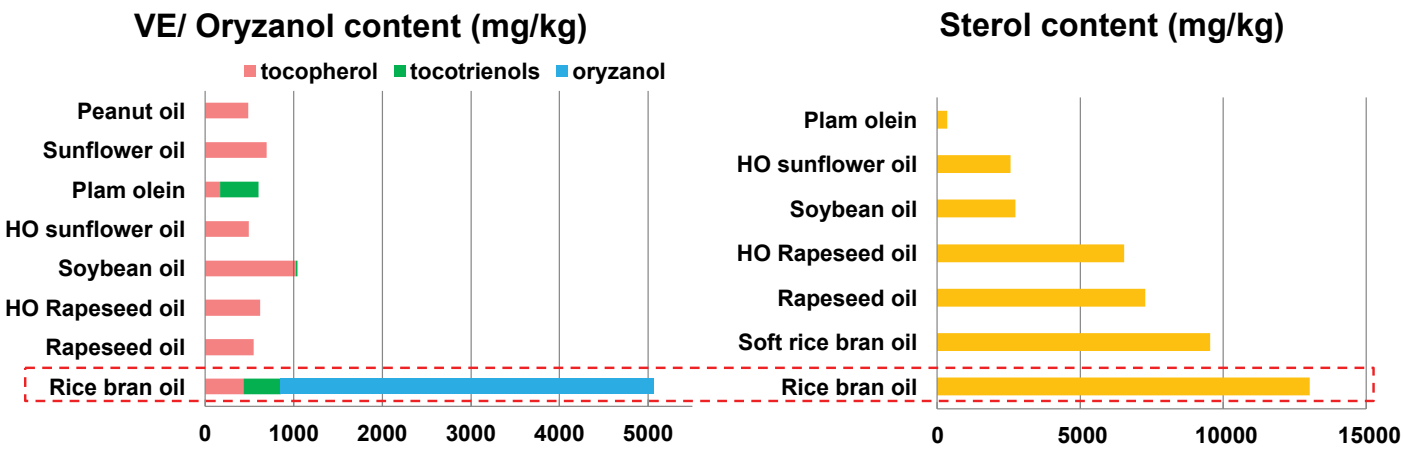


FIG. 4. Comparison of minor components in various oils. Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

transported to bigger facilities for extraction of RBO. This industry pattern can be summarized as "localized stabilization, centralized extraction, and refining." In the case of Wilmar (in Jiamusi, Heilongjiang, China), implementing this industry pattern lowered the AV by 30% (Fig.5).

Chemical and physical neutralization are two options during oil refining. In China, most RBO producers use physical neutralization to improve yield and micronutrients content. It is worth noting that lately the enzymatic method has been successfully applied in the industry. The mechanism of enzymatic refining is shown in Figure 6. With enzymatic neutralization, the AV in RBO decreases significantly from 27.2 mg KOH/g to 3 mg KOH/g. This promising technique is not legalized and commercialized in China yet. More research and legislative work are desired from both industry and the government of China.

MARKETING CHALLENGES

RBO has a very long tradition of use in the food industry and catering businesses in some Asian countries, such as Japan, India, and Thailand. In China, RBO is mostly consumed as a household culinary oil. Recently, more companies have begun to use RBO as an ingredient to enhance nutrition and performance of their products. As manufacturers become more familiar with RBO's functional advantages (e.g., enhanced frying stability, unique flavor after heating, and high viscosity), the popularity of RBO as a food ingredient will continue to grow among manufacturers and consumers.

Refined RBO has a subtle flavor at ambient temperature. After heating to 180°C, the flavor of the oil becomes nutty and sweet. The enhanced flavor upon heating could be due to the

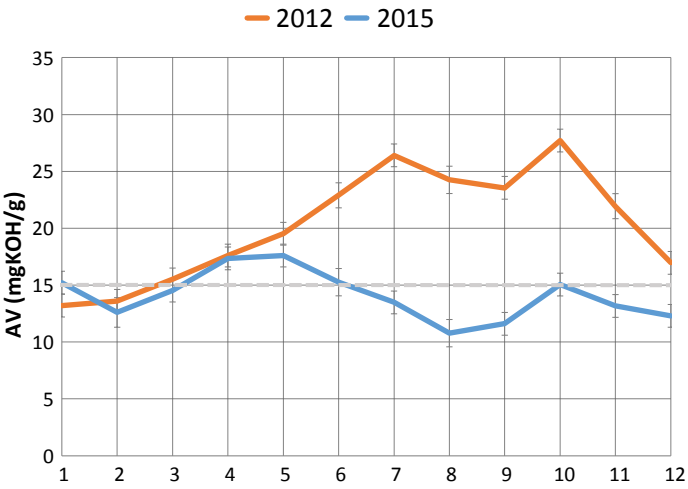


FIG.5. AV change of crude oil at Wilmar (Jia Mu Si) manufactory. Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

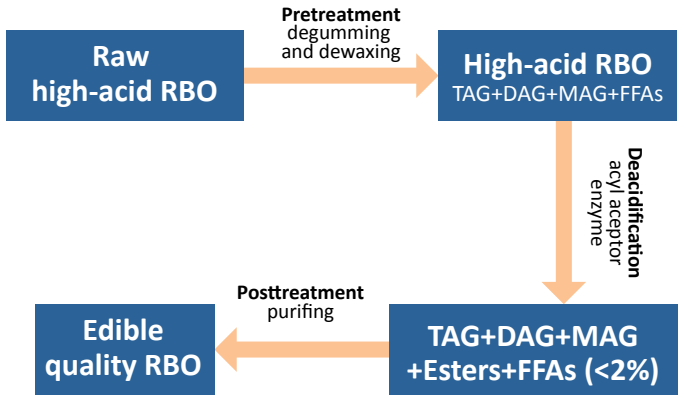


FIG. 6. Typical enzymatic refining. Source: Ghosh, 2007

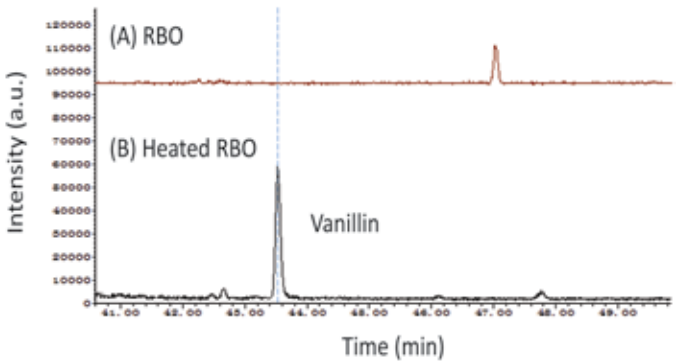


FIG. 7. Flavor compound analysis of RBO. Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

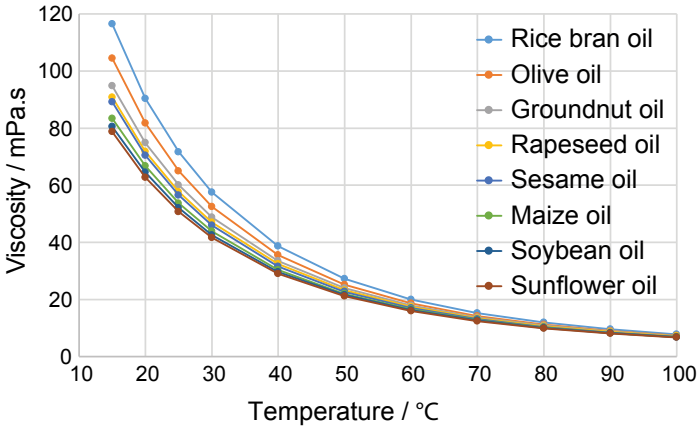


FIG. 8. Viscosity changes of various oils in different temperature.
Source: Wilmar (Shanghai) Biotechnology R&D Center Co., Ltd.

presence of vanillin during heating. Flavor compounds present in unheated and heated RBO (180°C) have been tested using GC-MS (Fig. 7). Interestingly, potato chips fried with RBO have been commercialized and are preferred by customers due to their enhanced flavor and crispiness.

In China, liquid vegetable oils are the major edible oils used for daily cooking, like stir-frying. For most stir-fried food, such as Chinese-style salads and Ramen, oils are expected to be viscous so that they cling to the surface of the foods and make them smoother. RBO has a higher viscosity than many other oils (Fig.8). This is an application advantage in the catering industry.

All in all, the industry in China has put great effort into RBO. Processing technologies, product quality, and edible applications have been developed, and RBO is now recognized as a primary edible oil by more and more consumers. The International Association of Rice Bran Oil (IARBO) was established in 2013 to develop and promote improved communications among RBO producers, academic researchers, and local governments. This non-profit international organization is dedicated to achieving world recognition of RBO by developing RBO production and its commercial application.

Corresponding author Yuanrong Jiang is the Deputy General Manager of Wilmar Global R&D Center. She received her Ph.D. degree in cereals, oils, and vegetable proteins from Jiangnan University, Wuxi, China. Her major research interests are fats and oils, including processing, byproducts application, flavor, lipids oxidation, and frying science. She also works on protein nutrition and food safety. She has published over 60 peer review journal papers and book chapters, and can be contacted at jiangyuanrong@cn.wilmar-intl.com.

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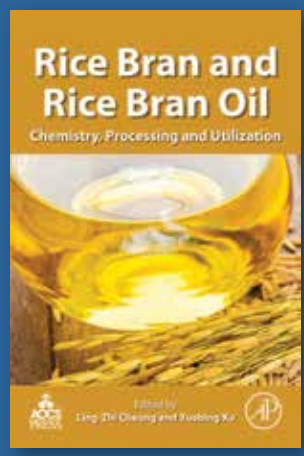
More on rice bran oil?



You can find the latest information in *Rice Bran and Rice Bran Oil—Chemistry, Processing and Utilization* (ed. Cheong, L.-Z. and Xu, X.), Elsevier, 2019. The contributors, who are all experts in their fields and include two authors of this article, "have been diligent in seeking out the latest information," according to Geoff Webster of Foodinc Food Industry Consultants in Auckland, New Zealand, who noted that "it is great to see the number of references to work published in 2017 and 2018," and that "the wide geographical spread of the contributors adds to the strength of this book." You can read his review of the book at https://www.aocs.org/store/shop-aocs/shop-aocs?productId=232365172.

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Spectroscopic on-line and real-time monitoring of edible oil refining

Jonathon D. Speed

- **Spectroscopic techniques (NMR, UV-vis, FTIR, NIR) are powerful analytical tools for increasing process efficiencies and resource utilization in the edible oil industry.**
- **Each established spectroscopic technique has limitations and strengths when adapting its use from the laboratory to on-line industrial environments.**
- **Improving the design of a mid-infrared (FTIR) spectroscopic instrument creates an on-line process analyzer that is robust enough to perform *in situ*, without sacrificing laboratory-grade analytical capabilities in the monitoring of edible oil refining processes.**

Off-line spectroscopic analysis of oils and other organic molecules is an established process widely used in industry and academia, from synthetic chemistry laboratories to high-end quality control analytical laboratories. The full range of the electromagnetic spectrum is used, and techniques such as nuclear magnetic resonance (NMR), ultra-violet visible (UV-vis) and fluorescence, and Fourier transform infrared (FTIR), have become commonplace. While off-line laboratory instruments are well-known and well-used, on-line instrumentation is rarer and more difficult to incorporate into process analysis, let alone process control—a crucial tool for "next generation" oil-refining processes. Vibrational spectroscopy (FTIR and NIR) are powerful techniques for oils analysis, because they study the molecular backbone of the oils and any contaminants, giving information on vibrating chemical bonds within the molecules. They can be used to study properties of oils (for example, the extent of oxidation) but also for fingerprinting different chemical concentrations within a mixture.

CHALLENGES OF ON-LINE SPECTROSCOPY

Various process analytical instruments have been brought to market based on vibrational spectroscopy over the last 20 years, but they all had various drawbacks or issues that have reduced their efficiency and effectiveness in real-world scenarios. Both conventional FTIR and NIR instruments use an array of moving mirrors to create the spectrum, which means that the body of the instrument must be removed from the process being investigated to prevent disturbance from pumps and similar machinery; this is generally achieved by using fiber-optic cables from the probe to the reaction. These fibers are frag-

ile and, in the case of FTIR, can be so sensitive to movement themselves that the instruments have very limited use in industrial environments.

Additionally, NIR instruments do not directly observe chemical bond vibrations, but instead can only detect overtones and combinations of vibrations. This means that the quality of information from an NIR instrument is inherently much lower than that of an FTIR, limiting the applications such instruments can add value to monitoring, as similar molecular structures have very similar spectra. For example, NIR instruments may struggle to differentiate different chain lengths in free fatty acids (FFAs) or to distinguish mono, di- and triglycerides. Also, NIR instruments require special transmission cells for sampling, and these cells can get blocked and be difficult to install into existing processes.

Therefore, to benefit from the spectral information of FTIR but resolve the underlying problems of instrument fragility, a novel and different approach is required.

OVERCOMING THE ISSUES WITH CONVENTIONAL FTIR

Through the use of static optics and a solid "light pipe" approach to a probe, we have created an instrument that yields the information quality of an FTIR spectrometer, but with dramatically improved robustness and reliability: the IRmadillo. A schematic of the optical layout is shown in Figure 1. The process involves the following:

1. Broadband infrared radiation is generated by the emitter at the end of the probe.
2. This is shone onto an attenuated total reflection (ATR) tip that is placed in the sample.
3. Some of the light escapes the ATR, interacts with the sample to be studied, and then returns back into the probe.
4. This light hits a beam splitter where half the light is allowed clockwise around a loop of mirrors.
5. The other half of the light runs counter-clockwise around the same loop.
6. Both beams of light hit the detector but are slightly offset, creating an interference pattern.
7. This pattern is then collected by the detector.
8. A Fourier transform is performed on the data to create a spectrum.

The static nature of the optics (i.e., no moving mirrors or gratings) means that if the instrument is subjected to vibration, the entire array vibrates as a monolithic block. This, in turn, allows the use of a solid light pipe instead of fibers, so the instrument itself can be bolted directly onto the process to be studied.

OIL MONITORING AND DETECTION LIMITS WITH NOVEL FTIR

To establish the benefits of using on-line monitoring for refining, a mixture of sunflower oil containing a variety of different FFAs and fatty acid methyl esters (FAMEs) was analyzed using the instrument. Variable mixtures of both FFA and FAME ensure that the analyser works regardless of the exact makeup of different chemicals. The total FFA values vary between 0 and 1.54%, and FAME values vary between 0 and 0.32% (Table 1, page 20).

The spectra obtained during calibration are shown in Figure 2. The underlying spectrum of oil does not change with

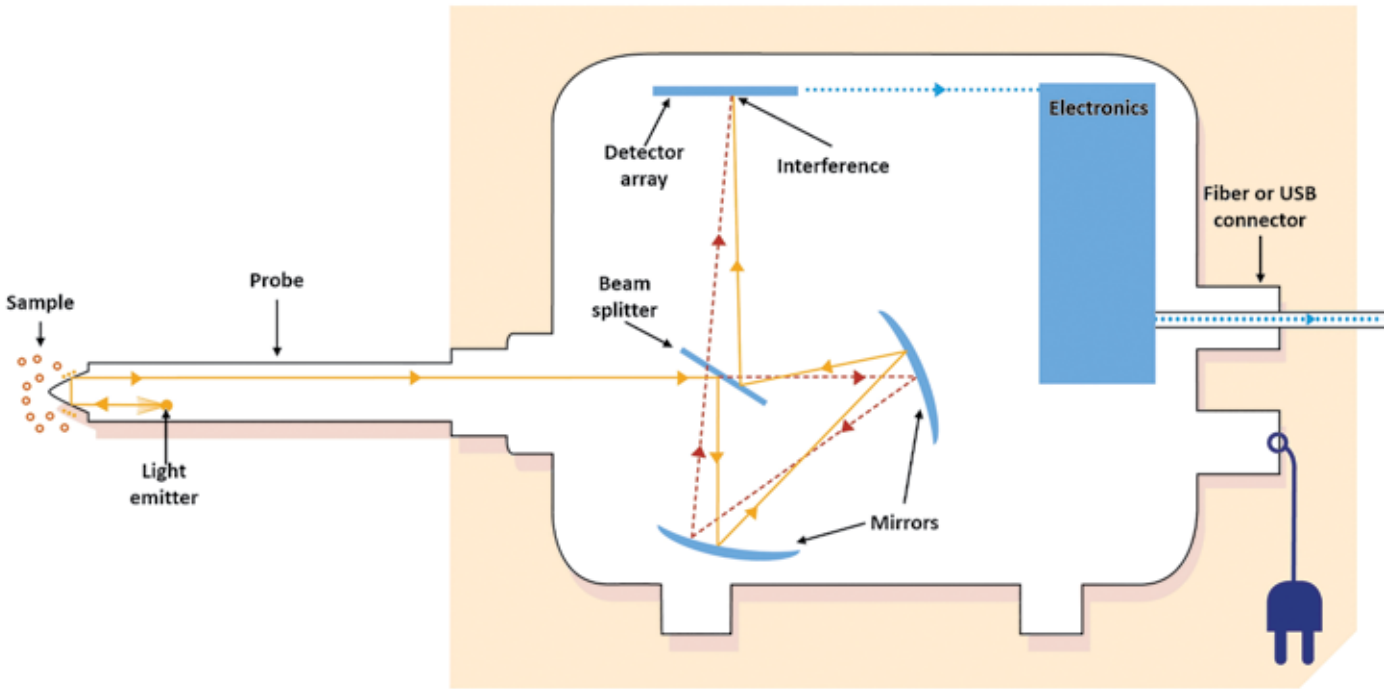

FIG. 1. Schematic of light path within cross section of the IRmadillo, a novel process FTIR spectrometer

TABLE 1. Mixtures used to calibrate total FFA and total FAME measurements

Palmitic acid / %	Stearic acid / %	Oleic acid / %	Linoleic acid / %	Linolenic acid / %	Methyl stearate / %	Methyl palmitate / %	Refined base sunflower oil / %	Total FFA / %	Total FAME / %
0.26	0.07	0.96	0.00	0.25	0.09	0.24	98.13	1.54	0.32
0.26	0.07	0.96	0.00	0.25	0.09	0.24	98.13	1.54	0.32
0.26	0.07	0.96	0.00	0.25	0.09	0.24	98.13	1.54	0.32
0.00	0.07	0.48	0.03	0.00	0.00	0.14	99.26	0.60	0.14
0.00	0.07	0.48	0.03	0.00	0.00	0.14	99.26	0.60	0.14
0.00	0.07	0.48	0.03	0.00	0.00	0.14	99.26	0.60	0.14
0.01	0.00	0.01	0.04	0.10	0.00	0.01	99.82	0.17	0.01
0.01	0.00	0.01	0.04	0.10	0.00	0.01	99.82	0.17	0.01
0.01	0.00	0.01	0.04	0.10	0.00	0.01	99.82	0.17	0.01
0.01	0.00	0.02	0.99	0.01	0.09	0.15	98.73	1.03	0.24
0.01	0.00	0.02	0.99	0.01	0.09	0.15	98.73	1.03	0.24
0.01	0.00	0.02	0.99	0.01	0.09	0.15	98.73	1.03	0.24
0.01	0.10	0.00	0.92	0.11	0.09	0.01	98.77	1.13	0.09
0.01	0.10	0.00	0.92	0.11	0.09	0.01	98.77	1.13	0.09
0.01	0.10	0.00	0.92	0.11	0.09	0.01	98.77	1.13	0.09
0.01	0.01	0.41	0.79	0.15	0.00	0.01	98.61	1.37	0.02
0.01	0.01	0.41	0.79	0.15	0.00	0.01	98.61	1.37	0.02
0.01	0.01	0.41	0.79	0.15	0.00	0.01	98.61	1.37	0.02
0.19	0.01	0.08	0.84	0.11	0.00	0.17	98.61	1.22	0.17
0.19	0.01	0.08	0.84	0.11	0.00	0.17	98.61	1.22	0.17
0.19	0.01	0.08	0.84	0.11	0.00	0.17	98.61	1.22	0.17
0.18	0.07	0.14	0.66	0.00	0.00	0.01	98.94	1.05	0.01
0.18	0.07	0.14	0.66	0.00	0.00	0.01	98.94	1.05	0.01
0.18	0.07	0.14	0.66	0.00	0.00	0.01	98.94	1.05	0.01
0.12	0.00	0.42	0.03	0.00	0.09	0.00	99.34	0.57	0.09
0.12	0.00	0.42	0.03	0.00	0.09	0.00	99.34	0.57	0.09
0.12	0.00	0.42	0.03	0.00	0.09	0.00	99.34	0.57	0.09
0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00

FFA and FAME level, but features across the spectrum clearly change between spectra. See the caption Figure 2 for band assignment.

By analyzing the spectra using chemometrics, a calibration curve can be built which allows quantitative interpretation of the spectra to give real-time FFA and FAME measurement. The outputs of a support vector regression (SVR) model are shown in Figure 3. The limits of detection (calculated from the root mean squared error of cross validation from the model–RMSECV) are 0.06 and 0.24% for FAME and FFA, respectively.

QUALITATIVE MEASUREMENTS CLASSIFYING OILS AND ADULTERATION MONITORING

It is very well known that lab instruments can monitor and differentiate between oils and spot adulteration; the literature is

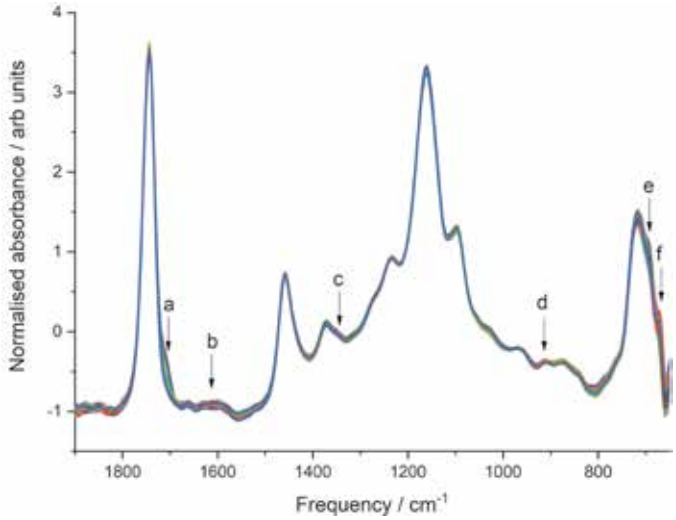

FIG. 2. Spectra acquired on the IRmadillo during calibration of FFA and FAME mixtures in sunflower oil. Spectra were acquired in triplicate on 120 s averaging times with a range of 648 to 1900 cm^{-1} at an interval of 4 cm^{-1} spacing. Band assignments are as follows: a) C=O in FFAs, b) C=C bonds in unsaturated FFAs and FAMEs, c) C-H bends in FFAs and FAMEs, d) C-C and C-O stretches in FFAs and FAMEs, e) O-C=O bend in FFAs, f) 0-C=O bends in FFAs and FAMEs.

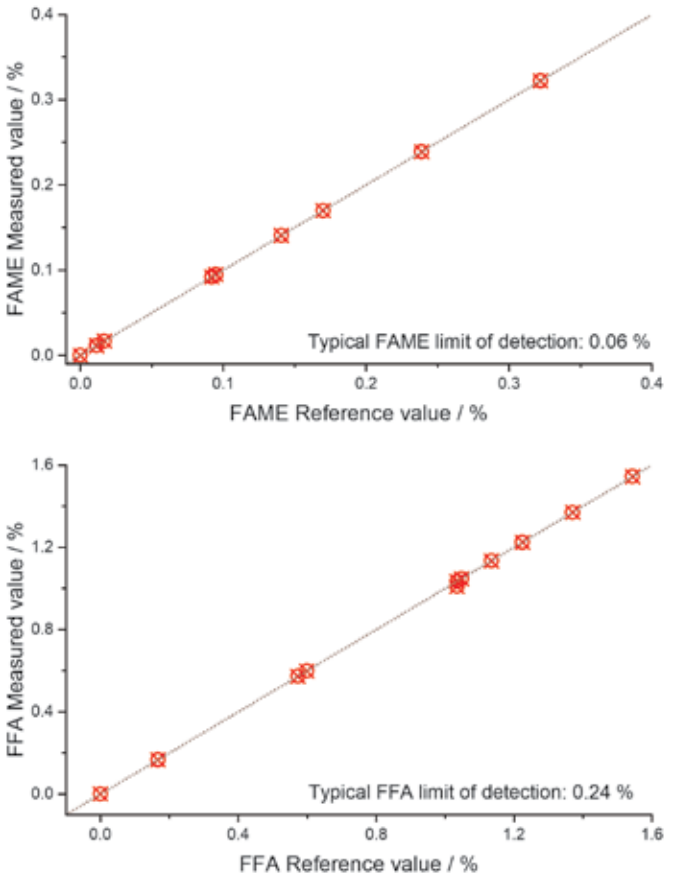

FIG. 3. Plots of calibration for the FAME and FFA measurements using support vector regression (SVR) chemometric modelling

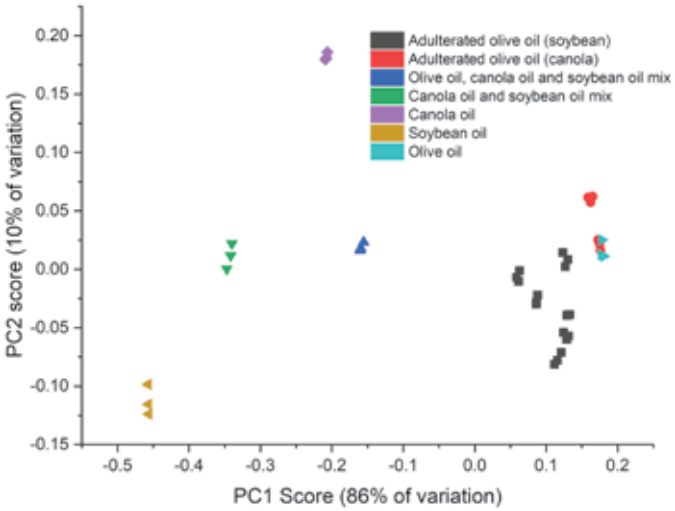

FIG. 4. PCA plot of scores for PC1 and PC2

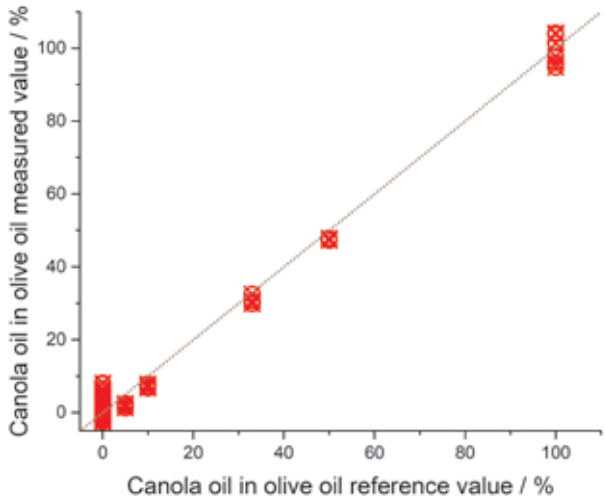

FIG. 5. Plot of calibration for canola oil in olive using PLS chemometric modeling

absolutely awash with examples. But performing this analysis with on-line, industrial instrument is much rarer. To evaluate the instrument's ability in this context, we analyzed various different oils and made intentionally adulterated samples of olive oil spiked with canola and soybean oil. These spectra were analyzed using principal component analysis (PCA), and the results are shown in Figure 4.

There are obvious clusters of different types of oil, making it easy to classify the different oil types. By using standard chemometrics tools (SIMCA or PLS-DA algorithms), a user can automate this process, having continuous analysis on an oil stream, easily spotting any divergence from the expected feedstock or adulteration. There is an overlap between the olive oil and adulterated olive oil (with canola oil). However, it is possible to quantify the amount of canola oil present within the olive oil—not only classifying that there is a blend, but also quantifying the degree of blending. This was performed using a partial least squares (PLS) model and is shown in Figure 5. The limit of detection (LoD) from RMSECV is 3.2%.

WHAT'S NEXT?

The nature of FTIR as an analytical technique also allows for many other molecules to be monitored and measured simultaneously. As a result, we also expect to use the instrument to measure:

- water,
- free glycerol,
- monoglycerides,
- diglycerides,
- soaps (as fatty acid sodium salts),
- phosphorus (as phospholipids – differentiating between hydratable and non-hydratable), and
- thermal degradation (oxidation)

We are actively seeking a partner in the industry to install the instrument directly onto a process line in the near future to gain much more data and move into real-time control of the refining process. Interested in seeing what this instrument can do at your facility? Please contact the author, Jonathon Speed at jonathon.speed@keit.co.uk.

Jonathon Speed is the Product and Applications Manager for Keit Spectrometers, Oxford, United Kingdom. He is an expert in vibrational spectroscopy with over a decade of experience in Raman and FTIR spectroscopies. He can be contacted at jonathon.speed@keit.co.uk.

Further reading

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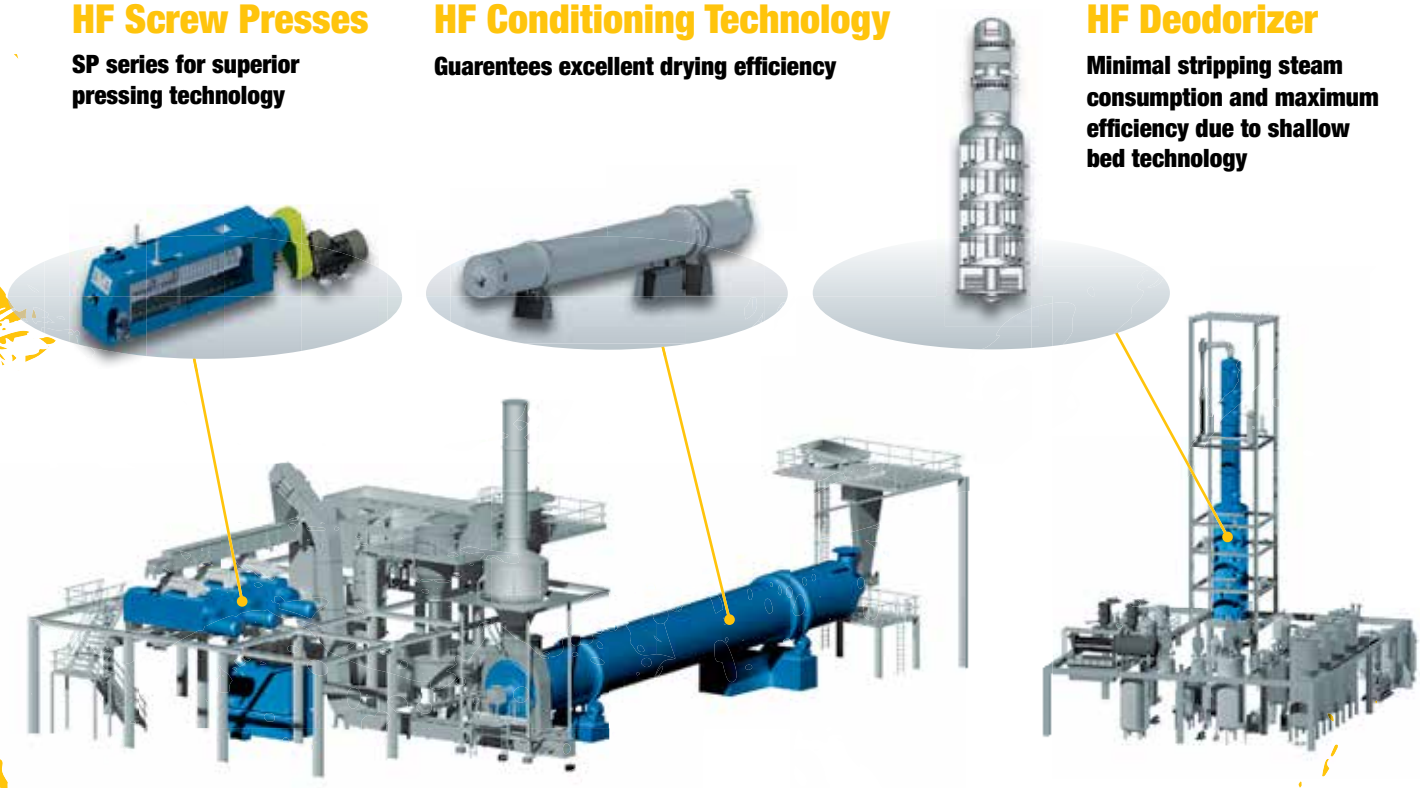

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Flavored rapeseed oil in China

Xiangyu Wang

According to the US Department of Agriculture, the annual rapeseed oil yield—27.97 million metric tons (MMT)—jumped to third highest among all oil species in 2018/2019, right after palm oil (74.08 MMT) and soybean oil (56.30 MMT) [1]. China is one of the biggest consumers of rapeseed oil: Domestic consumption for 2018 was 8.55 MMT, whereas domestic planting could only provide 4 MMT. Thus, half of the domestic demand for rapeseed oil must be met by imports. The rapeseed oil consumed in China can be divided into two main types: refined rapeseed oil and flavored rapeseed oil.

- More than 70% of the rapeseed grown in China is used to make flavored rapeseed oils.
- The volatile components in the oils are characteristic of the regions in which they are produced.
- This article discusses the two processing methods used to produce flavored rapeseed oil, and how each of those methods—and the volatile components in the oils from each region—contribute to their distinctive aroma and flavor.

Flavored rapeseed oil is popular in several regions of China due to its unique flavors. In 2018, the annual yield of flavored rapeseed oil in China was over 3 MMT. Over 70% of the domestic rapeseed were used to produce flavored rapeseed oil.

In China, the rapeseed plantations are distributed along the Yangze river, and can be divided into three geographic locations: upstream (Sichuan, Chongqing, Guizhou, Yunnan), midstream (Hubei, Hunan, Anhui), and downstream (Jiangsu, Zhejiang).

FIG. 1. Regional distribution of rapeseed oil production in China

The flavor of rapeseed oil produced upstream is different from that produced midstream and downstream. Flavored rapeseed oil produced upstream is characterized by a spicy aroma, whereas flavored rapeseed oil produced in midstream and downstream locations is less pronounced. Upstream rapeseed oil has a comparatively higher content of erucic acid and sulfur glucoside, which contributes to its distinctive aroma and flavor (Fig. 1).

Cold-pressing and roasting-pressing are two methods used to produce flavored rapeseed oil. Cold-pressing yields an oil with an astringent and slightly nutty flavor. It is generally extracted by a screw press at temperatures below 60°C. Roasting-pressing oil is prepared by roasting the rapeseed at 150–170°C for 20–40 minutes prior to extraction. This seed-roasting is the key step in producing rapeseed oil with a pleasant roasted flavor [2]. During the roasting process, the volatile compounds responsible for this roasting flavor are formed through the Maillard reaction, while Strecker degradation of glucosinolates and aldehydes generates an undesirable, pungent aroma [3]. After the roasting process, roasting-pressing oil is used to make flavored oil and refined oil. In the case of flavored oil, the roasted seeds are pressed, degummed, and filtered, after which most of the pleasant roasted flavor is retained in the oil. Refined canola oil goes through all the additional refining steps—extraction, degumming, neutralizing, bleaching, and deodorizing, during which odor- and flavor-producing compounds are removed.

The sensory properties of flavored rapeseed oils largely depend on their origin. The volatile components in flavored rapeseed oil included pyrazine, degradation products of sulfur glucoside, aldehydes, and heterocyclic compounds. The contents of key odorants from various flavored rapeseed oils are listed in Table 1.

TABLE 1. Key odor contents and their aroma intensity values from different rapeseed materials

Compound	Compound	Odor description	Content (%)			Aroma intensity value		
			Hubei	Sichuan	Canada	Hubei	Sichuan	Canada
Pyrazine	2,3-Dimethyl-pyrazine	Nutty, toasted	-	-	0.32	-	-	3.0
	2,5-Dimethyl-pyrazine	Nutty, toasted	1.81	2.11	7.19	1.7	2.4	4.1
	2-Ethyl-3-methyl-pyrazine	Burnt, toasted	0.47	0.42	1.94	1.0	0.9	3.3
	2-Ethyl-6-methyl-pyrazine	Nutty, toasted	0.78	0.66	1.97	1.3	1.1	3.7
	3-Ethyl-2,5-dimethyl-pyrazine	Nutty, toasted	0.76	0.79	3.13	0.8	0.6	2.0
Degradation of sulfur glucoside	5-Cyano-1-pentene	Spicy	7.59	15.94	2.56	3.9	4.8	2.2
	3-Butylene isothiocyanate	Spicy	5.39	13.05	1.13	3.1	4.6	1.7
Aldehydes	(E,E)-2,4-Heptadienal	Rancidity	0.39	0.19	0.44	1.2	0.8	1.3
	(E)-2-Octenal	Fatty, rancidity	-	-	0.35	-	-	1.5
	Nonanal	Fatty, spicy	2.09	-	0.74	2.1	-	0.8
	Heptanal	Fatty, rancid	-	-	0.11	-	-	-
	Hexanal	Herbal	-	-	0.95	-	-	1.5
Other heterocyclic	Butyl caprolactone	Toasted	-	1.94		-	-	2.2

-: Not detected.

Pyrazine is responsible for a nutty and toasted aroma [4, 5]. Compared to the rapeseed oil from Hubei and Sichuan, rapeseed oil from Canada preserves more pyrazine, including 2,3-dimetyl-pyrazine, 2,5-dimethyl-pyrazine, 2-ethyl-3-methyl-pyrazine, 2-ethyl-6-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, and the highest content of pyrazine is 2.5- dimethyl-pyrazine. The content of pyrazine from Canadian rapeseed oil is four times higher than that in Chinese rapeseed oil. Chinese rapeseed oil contains more 5-cyano-1-pentene and 3-butylene. These compounds are generated from the degradation of sulfur glucoside and provide a spicy aroma at higher concentrations. In contrast, Canadian rapeseed oil contains more degradation products of sulfur glucoside-(E,E)-2,4-heptadienal, and (E)-2-octenal. Aldehydes generated by lipid oxidation exhibit fatty, rancid, and herbal flavors. Butyl caprolactone creates a desirable aroma, resulting in a toasted odor that is only presented in Sichuan rapeseed oil.

The aroma intensity value is the average value of five sensory evaluators. Compounds with aroma intensity values >1 influence flavor markedly [2]. The aroma intensity values of degradation products of sulfur glucoside in Sichuan and Hubei rapeseed oil are higher than those in Canadian rapeseed oil, which is consistent with previous results of odor contents. In summary, the characteristic flavor of Sichuan and Hubei rapeseed oil is spicy, whereas Canadian rapeseed oil has a characteristic roasted flavor with higher aroma intensity values of pyrazine.

Xiangyu Wang is a researcher in food science and engineering at COFCO Nutrition and Health Research Institute, China. He can be contacted at wang_xiangyu@cofco.com.

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Restore skin lipids; improve overall health?

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

Rebecca Guenard

Like a suit of armor, human skin protects us against a daily bombardment of environmental attacks from pollution, pathogens, and electromagnetic radiation. But it only protects for so long. As humans age, our skin's resistance wanes. The skin's outer, most protective, layer, known as the stratum corneum, begins to decline after 50 years, no longer regulating moisture and restricting permeability as it once did (Fig. 1). New research published in the *Journal of European Academy of Dermatology and Venereology* suggests that these age-related changes may launch a systemic process that lurks beneath the epidermis and affects overall health (https://doi.org/10.1111/jdv.15540). Could restoring epidermal function reduce the chance of contracting the age-related diseases associated with this process?

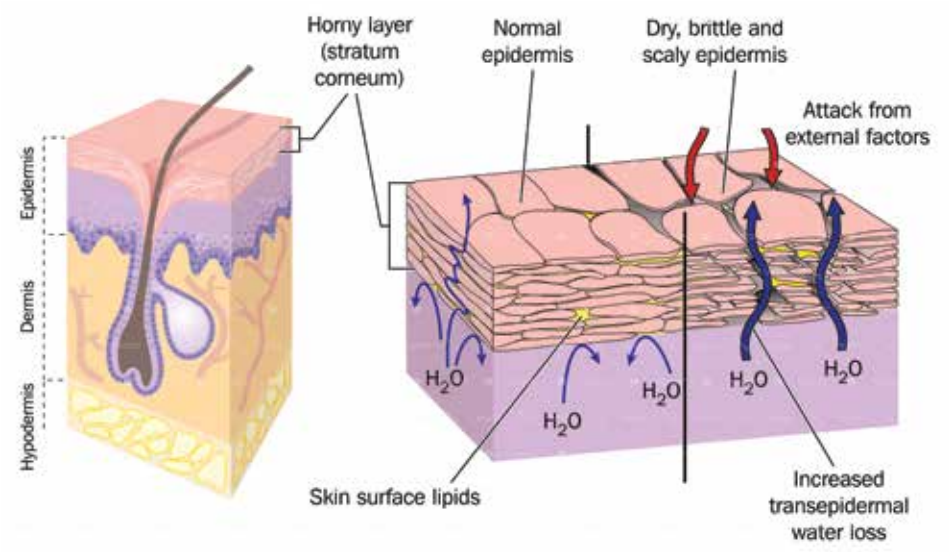

FIG. 1. Section through skin showing normal epidermis and skin surface structure resulting in water loss and dry, brittle skin.

"We want to try to maintain skin health as long as possible," says Anne Lynn Chang, associate professor of dermatology at Stanford University in Palo Alto, California, USA, who studies aging skin, but was not involved in the research. "That includes maintaining our skin barrier which is one of the primary defenses against the outside world."

To fulfill its protective role, the skin functions as a complex, dynamic system that incorporates multiple, interconnected biochemical processes, including pain sensing, immune response, and the metabolism of a lipid network. This network is composed of ceramides, cholesterol, and free fatty acids that stack in layers between epidermal cells in normal skin. In most skin disorders, such as atopic dermatitis, skin cells fail to synthesize and then secrete the full complement of lipids (https://doi.org/10.1159/000493641).

Mao-Qiang Man, a University of California, San Francisco (UCSF) research scientist and visiting professor at Southern Medical University in Guangzhou, China, has studied skin disorders for years and began to develop a hypothesis. He wondered if the epidermal abnormality that comes with age was related to a similar malfunction in lipid production that might, in turn produce a body-wide increase in inflammatory biomarkers.

For decades, researchers have been trying to determine why inflammation increases with age. Inflammation is the immune system's routine response to infection or injury. Receptors on immune cells launch a cascade of molecular responses, called cytokines, after sensing some form of harm or stress in the body. Scientist have proven that this beneficial system goes awry as we age, leading to an increase in inflam-

matory markers in the bloodstream. Over time, the constant presence of these molecules causes destruction and disease. Type II diabetes, cardiovascular and neurodegenerative diseases, rheumatoid arthritis, and cancers are all age-related diseases associate with inflammation (https://doi.org/10.3389/fimmu.2018.00586).

Man considered that the increased inflammation that occurs when people age did not involve a corresponding swelling or discomfort that is expected for a major immune response. He says a low-level immune response from a large organ is more logical.

"Then it comes to me that there are few organs or systems that are huge in the body," says Man. "One is the skin, others include the skeletal-muscular system, the intestine, the lungs. There are only a few huge organs."

As part of a team of dermatologists at UCSF and the San Francisco Veterans Administration (VA) Health Care, Man performed a study on 63 aged volunteers and 11 young volunteers with no known inflammatory disease. The researchers drew blood from all the participants to measure their cytokine levels. The aged group was then divided in half, and their entire skin surface was treated with a triple lipid formulation called Atopalm®, produced by Neopharm, a company in South Korea. They found that for three types of age-related cytokines the group that received Atopalm applications for 30 days had their cytokine levels cut in half, comparable to the level of the younger volunteers.

"There is a chronic barrier abnormality in aged skin beginning above age 50," says Peter Elias, a UCSF professor of dermatology who is based at the San Francisco VA Health Care System and part of the research team. He says when the barrier abnormality is chronic, it cannot be repaired, cytokines continually release in a futile attempt to restore the barrier, eventually leaking into the blood. "They never succeed, but if you use an appropriate formulation then you actually repair the barrier and shut off that cytokine cascade."

Elias and a team led by Theadora Mauro have performed several studies that are starting to piece together the crucial role of the skin barrier and how it changes with age. He advised Neopharm on their Atopalm® formulation after determining in previous studies inferior lipid production with age interrupts the skin's ability to maintain its moisture content. Without the proper amount of lipids in the right ratio, too much water can escape the skin. They found that moisturizers that coat the skin, like petroleum and lanolin, exacerbated the problem by blocking water transport, but lotions that substituted the missing lipids were restorative.

"There are three fats or lipids that are present in an approximate equimolar ratio (1:1:1), cholesterol, free fatty acids, and ceramides," says Elias. "You have to replace them at approximately the same ratio to do any good." He says that normal skin functions well on its own without need for treatment. But as we age, we produce fewer proteins that function as the enzymes that work to produce lipids. All three lipids decline, though the team found that cholesterol tapers off the most.

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Chang says this is an interesting and necessary study since not much dermatology research focuses on aging skin or how the body might respond internally to skin barrier damage. "There is not a lot of work on how these skin defects affect the whole body and health overall," she says. "Inflammation can have downstream effects when chronically overactive."

Man and Elias both point out that while their research indicates that lipid emollients applied to the skin lower cytokine levels in the blood, controlled clinical trials are need to evaluate the potential for treatment. They have begun developing mice models with cardiovascular disease to test if topical lipid emollients prevent or delay the disease's development. They also hope to explore the effect of lipid emollients on more types of inflammatory markers and to determine the amount of skin that needs to be treated to invoke a cytokine reduction.

"One thing that would be interesting to see is if repairing the barrier with an emollient improves the quality of life for patients, such as by decreasing itching and irritation" says Chang. Many aged patients report that their skin is itchy and painful. "If you can lower these cytokine levels does that also correlate with a reduction in clinical symptoms?"

Elisa says that all these immunologic effects physicians are treating are appearing in the skin and the blood because of the barrier abnormality. "It is not just arising as immunologic abnormalities associated with aging per se," he says. "They are arising because of a primary skin abnormality." With further research maybe corrective treatments will not only improve the skin barrier, but human health overall.

Olio is produced by Inform*'s associate editor, Rebecca Guenard. She can be contacted at rebecca.guenard@aocs.org.*

How are **producers addressing substances** of **concern?**

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

Leigh Stringer

The EU's REACH candidate list of substances of very high concern (SVHCs) has exceeded 200 chemicals. While an important milestone in regulating hazardous substances, it raises questions about how industry is addressing these and other chemicals of concern, particularly those who make or use them. To investigate, *Chemical Watch* asked 22 of the biggest global chemical companies:

- have they pledged to stop using SVHCs or "chemicals of concern" in the products they buy or sell;
- if yes, have they set a phase-out deadline? Which types of substances are to be phased out/avoided? For example, SVHCs, category 1&2 carcinogenic, mutagenic, reproductive toxicants (CMRs), persistent bioaccumulative toxic chemicals (PBTs), very persistent and very bioaccumulative *substances* (vPvBs), sensitizers, persistent organic pollutants (POPS), endocrine disruptors; and/or
- if no, do they plan to? And if not, why not?

The companies surveyed were chosen based on their market size and multinational status.

Of those surveyed, seven companies responded (Table 1). Only global nutrition, health and materials business Royal DSM had committed publicly to phasing out and avoiding chemicals based solely on their identification as substances of concern. In May, the company set a target to phase out all carcinogens; mutagens; reproductive toxicants (CMRs); and persistent, bioaccumulative, and toxic (PBTs) substances from products produced in its coating resins business by 2025.

Some NGOs have called DSM an industry leader for its approach. But what encouraged the company to take such action?

The company's decision makers believe that this is "future-proofing our business and our customers businesses," Sjoerd Dijkstra, sustainability marketer at DSM Coating Resins, said.

"Companies along the value chain, particularly brands, product manufacturers, and retailers, many of which we supply, are increasingly making these types of commitments on certain chemicals," Dijkstra said.

The guidelines and requirements that these downstream companies are putting forward to their suppliers can be very strict about certain chemicals, Dijkstra says.

Customers are asking DSM whether its products and materials can satisfy these requirements, so taking a similar position on chemicals of concern lays the groundwork for years to come.

This pressure is rippling through the value chain, starting with the consumer, and NGO campaigns. "There is a growing desire for disclosure of chemicals and ingredients. For only a few hundred dollars, a fragrance can be tested at a lab and the composition identified," Dijkstra said.

Legislation will follow this desire for chemical disclosure.

"The regulatory agenda is moving to a product level with a strong emphasis on transparency. And this fits with the circular economy concept, which, of course, will only become a more prominent piece of the business and regulatory jigsaw."

"TIME TO TAKE THIS SERIOUSLY"

DSM aims to be a purpose-led company.

"If you consider yourself a purpose-led and science-based company, with the aim of contributing to society in a positive way, then you need to conclude that the science is becoming evident that exposure to toxic chemicals is a severe risk to humans," Dijkstra said.

"As an industry, we have always thought risk is only a concern when you are exposed on a daily basis at very high doses. Now, the evidence is changing and showing that even micro-doses can be dangerous," he added.

"It is time industry took this issue seriously, and we invite peers to collaborate," Dijkstra said. "For example, you see some companies decide to sell part of the business that is under scrutiny because of chemicals of concern, but that is not going to change the market."

"To create this shift and transformation, as a leader you have to shout out publicly that this is not the direction we want to follow anymore."

BROADER CONSIDERATION

Other companies, such as Clariant, BASF, Dow and Lanxess provided statements on their approaches to chemicals of concern. Although the approaches vary, all apply broader considerations than simply a substance's classification to determine whether it should be phased out or avoided. Criteria considered during evaluations include social, environmental, and economic factors.

Evonik Industries said such a classification "does not automatically mean that any use of an SVHC is hazardous per se and has to be banned. It added that it "still applies the risk-based approach, taking exposure into consideration as well."

Dow says it is "important to recognize that SVHCs may not pose a true product safety concern, as this will be dependent on the specific applications, uses, and markets for the substance."

And Lanxess said that while it aims to minimize the harmful impact of chemicals on human health and the environment, certain production processes require the use of substances classified as SVHCs: "There are just no suitable alternatives to them. In those cases, our product stewardship covers the safe handling of these substances under strictly controlled conditions."

Dow's approach sets deadlines. The company's 2025 sustainability strategy includes a goal to evaluate its portfolio for "challenged chemistries" and set an approach for substitution, innovation, or stewardship. With this strategy, it plans to deliver 10 sustainable alternatives to the market by 2025. The company compiled criteria for what defines a sustainable alternative, which includes "performance; a favorable environmental, health, and safety (EHS) profile; enhanced lifecycle; and market acceptance."

Clariant screens its product portfolio against 36 criteria, which includes SVHCs as well as other non-desirable properties. Products containing SVHCs are typically categorized as "not sustainable."

However, they are not automatically earmarked for phase out. The company's next step is to develop a roadmap or action plan that can include efforts to replace an SVHC with existing alternatives, innovation, or ultimately to phase it out.

"Actions and timelines will depend on the opportunity to replace the substance, taking into account technical feasibility and regulatory context, which will depend on the substance and the particular geographical market. Therefore, some products may be SVHC, but still fulfil a need that currently no replacement can," Clariant said.

TABLE 1. The companies chosen for the survey were based on their market size and their multinational status.

Respondents to survey	Companies that did not respond	
• Arkema	• AkzoNobel	• ExxonMobil
• BASF	• Albemarle	• Honeywell
• Clariant	• Bayer	• Huntsman
• DOW	• Borealis	• Johnson Matthey
• DSM	• Celanese	• LyondellBasell
• Evonik	• Covestro	• Solvay
• Lanxess	• DuPont	• 3M
	• Eastman	

BASF'S APPROACH

Under BASF's approach, SVHCs, CMRs, and PBTs are identified but are not automatically set for substitution. Instead, they undergo an "impact check."

Along with the classification of the substance (SVHC, CMR, and so on), the impact check considers other factors including use and exposure. The next step could see a substance, for example, a CMR, classified as "challenged" and it would then be substituted within five years.

However, the fact that a substance is an SVHC, CMR, or PBT does not automatically place it in the challenged category. Other factors come into consideration, specifically use and/or exposure, but also "urgency" (time pressure) and "challenge of remediating." Urgency would include regulatory timelines, such as sunset dates set under REACH authorization.

Overall, BASF does not commit to remove/avoid all identified SVHCs. Like others, it considers these substances alongside a number of other factors.

What is consistent among the survey respondents, is that the majority have announced their approaches in the last two years, which shows that the topic of SVHCs and substitution has risen up the corporate agenda. However, while momentum is building, the survey also shows that a large number of chemical companies have either not yet publicly announced a strategy or are not willing to engage in a discussion on phasing out and substituting such chemicals.

"SEVERAL YEARS"

Cefic said the industry is not avoiding such a discussion.

"It is not surprising that some companies are not vocal about their substitution strategies. Substitution typically offers companies a competitive advantage. Therefore, they don't want their competitors to have a hint of the direction in which their research and development efforts are going.

"Not being vocal does not mean companies are not doing any substitution. They are."

Cefic said its members are working on substitution every day to make new substances or improve the properties of existing ones to better serve market needs.

"What is important to understand about substitution is that we can't use a cookie-cutter approach to all SVHC cases. Developing a substitute is an extremely complex and time-consuming process," it said.

"Ultimately, a substitute is only successful if it performs the same function as the original substance and at the same time has a less adverse impact on health or environment.

"If we want informed and not regrettable substitution, we have to accept that doing a proper job can take several years," Cefic said.

Leigh Stringer is Global Business Editor for Chemical Watch.

Creating a far-flung Section

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

After gaining a Ph.D. from the National University of Singapore in 2015, Sui Xiaonan returned to his original institution—Northeast Agricultural University in Harbin—to fulfill his childhood dream of being a teaching scientist. There, his research focuses on soybean and soybean co-products in large part because his hometown of Heilongjiang is in China's major soybean production area.

Sui Xiaonan's main role in AOCS is as secretary-general of the China Section, which was established in April 2016. "My main responsibility is to promote the connections between AOCS and China," he explains. "As such, we hold a China Section technical conference in China every two years." The 2019 meeting will be held in Guangzhou on November 8–10 (see information box).

As is the case with many AOCS volunteer leaders, Sui Xiaonan's involvement came about by being in the right place at the right time. "In 2014, Jiang Lianzhou and I attended the AOCS AM&E for the second time, where we met our friend Liu Keshun [Keshun Liu] during the gala dinner. It was then that we agreed on the necessity of creating the AOCS China Section."

The challenges of the new China Section include building effective connections among foreign and domestic experts, he notes, as well as between industry and academia. He adds that the mission of the Section is multi-faceted and includes: 1) promoting information exchange, collaboration, and partnership among the Chinese and global fats, oils, and related industries; 2) bringing together professionals in the geographical region; 3) supporting friendship among and the professional growth of its members; and 4) ensuring adherence to high standards, superior quality, safety, and value-added applications of fats, oils, proteins, and related materials.

"Membership in the China Section helps me to network with colleagues and discover scientific, technological, and market developments in this important region of the world," Sui Xiaonan concludes. "More importantly, it offers me the opportunity to learn from many famous experts and other AOCS members."

Fast facts

Name	Sui Xiaonan
Joined AOCS	2012
Education	Ph.D., food science and technology, National University of Singapore (2015)
Job title	Professor
Employer	College of Food Science, Northeast Agricultural University (Harbin, China)
Role in AOCS	Secretary-General, China Section
High-fat Indulgence	Harbin's sausage, a notable local product that tends to be of a much more European flavor than other Chinese sausages
Favorite Social Media	ResearchGate, Google Scholar, LinkedIn
Most memorable AOCS experience	"My first time chairing a session at an AOCS Annual Meeting & Expo (AM&E) was in 2018. It was a precious time to gain knowledge from plant protein experts."
Other involvement	AOCS member

2019 AOCS China Section Conference

The two-day **AOCS China Section Conference: Health, Advanced Processing, and Value-Added Utilization, November 8–10, 2019, http://www.aocschina.com/aocs/index.shtml**, will be held at the **Zhujiang (Pearl River) Hotel,** Guangzhou (Canton), China, a city in Southern China, not far from Hong Kong and Shenzhen. A half-day plenary session will cover raw material production and supply; marketing trends and consumer perceptions; special oilseed crops and lipids; health and nutrition; and advanced processing and applications for lipids, proteins, and co-products. A full day of technical sessions will cover analytics, lipid oxidation, safety, lipid oxidation, quality, biocatalysis, processing, edible and industrial applications, health benefits, biopeptides, and proteins. Young scientist forums and site visits to two nearby universities are also on the agenda.

Peru: stop sign labeling law in effect

Leslie Kleiner

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

On June 17, 2019, Peru implemented modifications to its "Ley de Alimentación Saludable (Law of Healthy Foods). The law now implements a labeling modification, which takes place after a six-month transition period starting in June. With these new modifications, solid foods and beverages with high levels of sodium, sugar, and saturated fats must now have an octagon on the front package with text warning that the contents are "high in" any of these ingredients. The law resembles the labeling strategy in Chile, which has been in place for over three years.

Q: What does "high in" mean, in terms of labeling?

"High in" labels would be as follows during the first phase of the implementation [1,2]:

- Sodium (solid foods), more than or equal to 800mg /100g
- Sodium (beverages), more than or equal to 100mg/100ml
- Sugar (solid foods), more than or equal to 22.5g /100g
- Sugar (beverages), more than or equal to 6g/100ml
- Saturated Fats (solid foods), more than or equal to 6g /100g
- Saturated Fats (beverages), more than or equal to 3g /100g
- Trans fatty acids (according to separate law)

At 39 months of the implementation of the law, the above parameters would become stricter and be as follows:

- Sodium (solid foods), more than or equal to 400mg /100g
- Sodium (beverages), more than or equal to 100mg/100ml
- Sugar (solid foods), more than or equal to 10g / 100g
- Sugar (beverages), more than or equal to 5g/100ml
- Saturated Fats (solid foods), more than or equal to 4g /100g
- Saturated Fats (beverages), more than or equal to 3g / 100g
- Trans fatty acids (according to separate law)

Q: Are there any exceptions?

The octagon must be placed in all processed food and beverages in which the package measures at least 50 cm^2, which by default excludes individual packages of many small products

(e.g., candy) but not bulk packaging. However, the Minister of Health, Zulema Tomás, pointed out that there is an ongoing effort to reduce the packaging size limitation in order to broaden the scope of products [3]. The octagons will not be applicable to food ingredients (defined as those that are minimally or not processed), such as vegetable oils, sugars, honey, salt, and flours. These ingredients are usually used at home to prepare meals [4].

Q: What would the new minimum package size, transitioning out of the 50 cm², be?

Gustavo Rosell, General Manager Strategic Interventions in Public Heath, Health Ministry, shared that the change would be progressive enough for the industry to adopt. He also mentioned that one of the objectives of this law is to promote portion and packaging size reduction. The impact of the current law would be measured in 6 months to a year. Based on the data, it would be determined if a minimum package of 30 or 20 cm² would be required [3].

Q: Are there sanctions if the octagons are not included in the labels?

Sanctions can range from a letter to a fee ranging from 700 UIT [3] to 10% of the gross income of the entity at fault [4]. UIT is the "Unidad Impositiva Tributaria" (Tax Unit), which is determined by the Ministry of Economy utilizing various macroeconomic parameters. This Tax Unit is a reference parameter that is usually applied to tax law and fees [5]. However, other sanctions and corrective actions, such as product recalls and destruction, non-permanent operation shut down, and others are also possible [4].

Q: What will the octagons say, and must the message be included in commercial ads?

The octagons will have the following message for high fat, sugar, or sodium products: "Avoid excessive consumption." For high trans-fat products, the message will say: "Avoid consumption." The warnings are not only for packaging labels, but they must appear in TV and radio ads for such products, and in any other commercial add. For written media and Internet, the size of each warning label must be at least 3.75% as big as the size of the add. In other words, advertisements for products that are high in all four ingredients (salt, sugar, saturated fat, and trans fat) would require four octagons that each at least 3.75% as big as the size of the ad, with the warnings taking up 15% of the space for the ad. For radio or other audio-related media, the label is to be read at the same volume and speed as the rest of the add, and its duration will be proportional to the add [5].

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22nd International Symposium on Surfactants in Solution

Journal of Surfactants and Detergents (JSD) Special Issue

The September 2019 issue of the *Journal of Surfactants and Detergents (JSD)* represents papers presented at the 22nd International Symposium on Surfactants in Solution (SIS-2018), June 3–8, 2018, on the University of Oklahoma in Norman campus. About 150 scientists from all over the world gathered for the meeting, which was hosted by the Institute of Applied Surfactant Research.

The biennial SIS meetings are considered the premier forum for the latest research and technology. Nearly 25 JSD editorial board members attended, including Editor-in-Chief George Smith and Senior Associate Editors Nancy Falk and Carlos Rodriguez Abreu.

The cross-section of papers presented in the SIS special issue is a great introduction to the state of surfactant research in 2018.

The 25 contributions represent 11 countries. As is true in the surfactant industry in general, the hydrophilic-lipophilic difference equation is well-represented, with one review paper and three articles primarily concerned with this approach. The editors aimed to provide a good balance between experimental and computational work.

Following are the contents:

Viscoelastic Behavior of Alkyl Ether Sulfate Systems Containing Nano-sized Colloidal Silica
Adamy, Steven

Observing the Effects of Temperature and Surface Roughness on Cetyltrimethylammonium Bromide Adsorption Using Quartz-Crystal Microbalance with Dissipation Monitoring
Grady, Brian

Characteristics of polypeptide/phospholipid monolayers on water and the plasma activated PEEK support
Jurak, Małgorzata

Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behaviour of phospholipid SOPC
Genova, Julia

Surfactant crystals as stimulable foam stabilizers: tuning stability with counter-ions
Salonen, Anniina

Aqueous foam stabilized by hydrophobic SiO_2 nanoparticles using mixed anionic surfactant systems in a high salinity-brine condition
Rattanaudom, Pattamas

SHORT COMMUNICATION

On the relationships between the hydrophilic-lipophilic balance and the nanoarchitecture of nonionic surfactant systems
Rodriguez-Abreu, Carlos

LETTERS TO THE EDITOR

New molecular descriptors to identify surfactants and solubilizers from electron density distributions
Gaudin, Théophile

Carbamate chemistry at interfaces: Practical considerations and challenges of studying amine surfactants
Hellström, Anna-Karin

Thank you George Smith, Editor-in-Chief, 2015–2019

AOCS appreciates the vision, leadership, editorial work, and many hours of service to the *Journal of Surfactants and Detergents* Smith provided while serving as Editor-in-Chief for the past five years. Pam Landman, AOCS Journals Coordinator comments on the direction he set during his term:

"JSD is still a relatively young journal in the field, and George has helped to build its legacy as a journal that strives to meet the needs of researchers in both the theoretical and applied sciences. Also, I will miss his dry, to the point of dusty, sense of humor."

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PATENTS

Method for producing fat and oil composition

Saito, K., *et al.*, Kao Corp, US10231468, March 19, 2019

Provided is a production method which can produce, with a high yield, a fat or oil composition containing a diacylglycerol at a high content and having a favorable external appearance at room temperature. Specifically, provided is a production method for a refined fat or oil composition comprising the following steps (1) and (2): (1) a step of adding a polyglycerin fatty acid ester having an average polymerization degree of glycerin being 20 or more to a fat or oil composition containing 50 mass % or more of diacylglycerol, followed by cooling; and (2) a step of separating a crystal precipitated in the step (1) from a liquid portion.

Enzyme detection device

Davis, P., *et al.*, Mologic Ltd., US10234457, March 19, 2019

Described herein is an enzyme detection device for use in the detection of enzyme activity in a test sample. Also provided are indicator molecules for use in the detection of enzyme activity, particularly enzyme cleavage activity, in a test sample, and to methods for detecting the presence of enzyme activity.

Composition and method of using the same

Rath, M.W., *et al.*, US10238619, March 26, 2019

A composition of a fatty acids, essential oils, plant extracts, phenols, and terpenoids for treating Lyme disease is disclosed. Compositions as a mix of ingredients was tested in various combinations and concentration against active (spirochetes), as well as persistent/latent forms, (knob-shaped/rounded-forms) and biofilm of the bacteria, i.e., *Borrelia burgdorferi* and *Borrelia garinii*. The results document pleiotropic effects of the tested combinations against all tested forms of *Borrelia* sp. and show their better efficacy than doxycycline—the current antibiotic treatment against Lyme disease.

Method for producing high-purity omega-3 fatty acid ethyl ester

Shimizu, Y., *et al.*, Bizen Chemical Co., Ltd., US10240172, March 26, 2019

The present invention relates to the field of methods for purifying fatty acid ethyl esters. According to the present invention, a method for obtaining an omega-3 fatty acid ethyl ester, such as EPA and DHA, each as a high-purity product at a high yield is provided. In the method according to the present invention, a raw material fat including EPA and DHA is treated with a lipolytic enzyme and ethyl-esterification is performed as needed; the treated substance is fractionated into a glyceride fraction and a free fatty acid fraction; a fraction comprising more EPA ester and a fraction comprising DHA ester are obtained from the respective fractions; the fraction comprising more EPA ester is purified to prepare a high-purity EPA ester; and the fraction comprising more DHA ester is purified to prepare a high-purity DHA ester.

Frying machine

Miyoshi, O., Foods Tec Ltd., US10244896, April 2, 2019

A frying machine for efficiently placing ingredients into a frying basket and removing fried food from the frying basket. Ingredients are placed into and fried food is removed from a frying basket by moving the frying basket and a frying tank cover to above the frying tank using a first transfer mechanism, moving the frying basket and frying tank cover to a predetermined position using a second transfer mechanism, and causing the frying basket to descend to an ingredient input position or fried food output position using the first transfer mechanism. The ingredients are placed into the frying basket, and the fried food is removed from the frying basket efficiently at the ingredient input position or fried food output position separated in the horizontal direction from a position above the frying tank in a configuration equipped with the frying tank cover adapted to open and close the frying tank.

Method of treating cancer with edible-oil-derived excipients

Lele, B.S., US10245234, April 2, 2019

Present invention teaches an improved method of treating cancer comprising intravenously administering to a subject in need thereof substantially ethanol-free nanoparticles comprising an anticancer drug and a substantially hydroxyl free unsaturated edible oil saturated at least at one site of unsaturation by a covalently attached hydrogen atom and a covalently attached water-soluble polymer.

Plasticizer for color retention during heat aging

Mundra, M.K., *et al.*, Dow Global Technologies LLC, US10262767, April 16, 2019

The present disclosure is directed to a plasticizer, a polymeric composition containing the plasticizer, and conductors coated with the polymeric composition. The plasticizer includes a blend of an epoxidized soybean oil and a trans-esterified epoxidized fatty acid methyl ester. The transesterified epoxidized fatty acid methyl ester has an oxirane value greater than or equal to 6.8%. The plasticizer, the polymeric composition, and the coated conductor retain color during heat aging.

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

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ANA Analytical
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H&N Health and Nutrition
IOP Industrial Oil Products
PRO Processing
PCP Protein and Co-Products
S&D Surfactants and Detergents

ANA Determination of neonicotinoids and 199 other pesticide residues in honey by liquid and gas chromatography coupled with tandem mass spectrometry

Gaweł, M., *et al.*, *Food Chem. 282*: 36–47, 2019, https://doi.org/10.1016/j.foodchem.2019.01.003.

The development of sophisticated analytical techniques can detect what was previously undetectable. This report identifies a whole group of pesticides and residues in honey. The methods can also be used to detect similar compounds in other natural products.

Current work presents a modified QuEChERS method for the determination of 207 pesticide residues in honey by LC–MS/MS and GC–MS/MS. Acetate-buffered acetonitrile extraction with Z-Sep+ and PSA dispersive-SPE clean-up were used for sample preparation. Optimized conditions allowed determination of neonicotinoids as well as other insecticides, fungicides, herbicides, acaricides, growth regulators, and veterinary drugs in honey samples. Validated method enabled sensitive analysis at concentrations from 0.001, 0.005 or 0.01 mg/kg for 45%, 41%, and 14% of pesticides, respectively. Method was utilized for the analysis of 155 honey samples from Poland during 2015–2017. Residues of 21 pesticides were determined in honey. Cyano-substituted neonicotinoids (acetamiprid, thiacloprid) were quantified in 77% of samples and were the most frequently detected pesticides. Concentrations of acetamiprid was from 0.001 to 0.13 mg/kg whilst thiacloprid from 0.001 to 0.2 mg/kg. Fungicides were determined in 50% and amitraz metabolites in 35% of honey samples.

ANA Chemical barcoding: a nuclear-magnetic-resonance-based approach to ensure the quality and safety of natural ingredients

Martinez-Farina, C.F., *et al.*, *J. Agric. Food Chem. 67*: 7765–7774, 2019, https://doi.org/10.1021/acs.jafc.9b01066.

This report leverages nuclear magnetic resonance (NMR) to authenticate natural products. Compared to other technologies, NMR is readily available in food labs and does not require radioactive carbon. However, there is still a need to develop a robust method for testing the identity of natural products that can be used by anyone.

One of the greatest challenges facing the functional food and natural health product (NHP) industries is sourcing high-quality, functional, natural ingredients for their finished products. Unfortunately, the lack of ingredient standards, modernized analytical methodologies, and industry oversight creates the potential for low-quality and, in some cases, deliberate adulteration of ingredients. By exploring a diverse library of NHPs provided by the independent certification organization ISURA, we demonstrated that nuclear magnetic resonance (NMR) spectroscopy provides an innovative solution to authenticate botanicals and warrant the quality and safety of processed foods and manufactured functional ingredients. Two-dimensional NMR experiments were shown to be a robust and reproducible approach to capture the content of complex chemical mixtures, while a binary normalization step allows for emphasizing the chemical diversity in each sample, and unsupervised statistical methodologies provide key advantages to classify, authenticate, and highlight the potential presence of additives and adulterants.

ANA EAT Nuclear magnetic resonance (NMR) spectroscopy in food science: a comprehensive review

Hatzakis, E., *Compr. Rev. Food Sci. F. 18*: 189–220, 2019, https://doi.org/10.1111/1541-4337.12408.

Nuclear magnetic resonance (NMR) spectroscopy is a robust method which can rapidly analyze mixtures at the molecular level without requiring separation and/or purification steps, making it ideal for applications in food science. Despite its increasing popularity among food scientists, NMR is still an underutilized methodology in this area, mainly due to its high cost, relatively low sensitivity, and the lack of NMR expertise by many food scientists. The aim of this review is to help bridge the knowledge gap that may exist when attempting to apply NMR methodologies to the field of food science. We begin by covering the basic principles required to apply NMR to the study of foods and nutrients. A description of the discipline of chemometrics is provided, as the combination of NMR with multivariate statistical analysis is a powerful approach for addressing modern challenges in food science. Furthermore, a comprehensive overview of recent and key applications in the areas of compositional analysis, food authentication, quality control, and

human nutrition is provided. In addition to standard NMR techniques, more sophisticated NMR applications are also presented, although limitations, gaps, and potentials are discussed. We hope this review will help scientists gain some of the knowledge required to apply the powerful methodology of NMR to the rich and diverse field of food science.

ANA Identification of new bioactive peptides from Kefir milk through proteopeptidomics bioprospection of antihypertensive molecules

Amorim, F.G., *et al.*, *Food Chem. 282*: 109–119, 2019, https://doi.org/10.1016/j.foodchem.2019.01.010.

Kefir, a probiotic beverage prepared from fermented milk, has been associated with antihypertensive activity. However, the bioactive molecules responsible for this activity remain unclear. Therefore, in this study we aim to evaluate the mechanisms of the antihypertensive effects of Kefir in the two-kidney one-clip hypertension model, and to bioprospect for bioactive peptides identified by proteomic methodologies. Treatment with Kefir was performed via gavage and resulted in a 37 mmHg reduction in systolic arterial pressure and 19% inhibition of angiotensin converting enzyme (ACE) activity. For the proteopeptidomic study, the protein extract of Kefir beverage and non-fermented bovine milk were analyszd by MALDI-TOF mass spectrometry, and their tryptic digestion products sequenced via shotgun proteomics (Q-Exactive mass spectrometer). A list of 35 peptides with potential hypertensive activity due to ACE inhibition were identified. These results demonstrate the benefits of Kefir products, and may guide the design of new antihypertensive drugs.

ANA LOQ Application of essential oils as a natural and alternate method for inhibiting and inducing the sprouting of potato tubers

Shukla, S., *et al.*, *Food Chem. 284*: 171–179, 2019, https://doi.org/10.1016/j.foodchem.2019.01.079.

Greater understanding of the different roles of essential oils and their individual components may lead to new natural preservation strategies in the personal care products industry.

Use of harmful chemicals and expensive maintenance of cold-storage conditions to control sprouting are among the major problems in potato storage. Here, 20 essential oils (EOs) were tested for their sprouting-inhibiting and sprouting-inducing activities. Overall, treatments of lemon grass (LG) and clove (CL) oils could induce sprouting, whereas palmarosa (PR) and ajwain (AZ) oils could inhibit sprouting of potato tubers at normal room-temperature (25 ± 2°C) storage. Selected-EOs treatments affected sprouting by modulating accumulation of reducing sugars, ethylene, and expression of genes involved in tuber-sprouting such as ARF, ARP, AIP, and ERF. Surprisingly, 7 days of AZ-treatments could inhibit sprouting for 30 days, which was mediated via damaging apical meristem. However, LG- and CL-treated tubers could produce enhanced potato yield as well. The present work clearly demonstrates that selected EOs can be used as a promising ecofriendly approach for inducing/inhibiting sprouting of potato tubers during potato storage and those enhancing sprouting can be used for enhancing productivity.

ANA H&N Anti-alcoholic effects of honeys from different floral origins and their correlation with honey chemical compositions

Guo, P., *et al.*, *Food Chem. 286*: 608–615, 2019, https://doi.org/10.1016/j.foodchem.2019.02.058.

This study demonstrated that the quality and metabolic effects of honey depend strongly on the flowers available to the bees. One can extend the logic to surmise that the chemical composition of micronutrients from crops cultivated in different region of the world may have minor differences, and that these differences may play a role in maintaining human health or otherwise. Therefore, it is important to know the origin of your natural products.

The effects of honeys from different floral origins on alcohol metabolism were compared, and the correlation between their chemical compositions and anti-alcholic effects was analyzed. The results demonstrated that the five types of investigated honeys from different floral origins had different effects on alcohol metabolism, and the blood alcohol removal rate by these honeys ranged from 18.01% to 49.17%. *Ziziphus jujuba* honey exhibited the best blood alcohol removal effect, and meanwhile significantly enhanced the activity of alcohol-metabolizing enzymes including alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Chemical composi-

tion analysis also showed that honeys from different floral origins were considerably different in the contents of sugars, minerals, ascorbic acid, and phenolics. *Ziziphus jujuba* honey had the highest fructose/glucose ratio, ascorbic acid and phenolics contents, and higher contents of minerals, especially K, Ca, Mg, Fe, Cu, Zn, and Mn. This chemical composition might contribute to its better anti-alcoholic effect.

BIO PCP PRO Mechanism-guided design of highly efficient protein secretion and lipid conversion for biomanufacturing and biorefining

Xie, S., *Adv. Sci. 6*: 1801980, 2019, https://doi.org/10.1002/advs.201801980.

Bacterial protein secretion represents a significant challenge in biotechnology, which is essential for the cost-effective production of therapeutics, enzymes, and other functional proteins. Here, it is demonstrated that proteomics-guided engineering of transcription, translation, secretion, and folding of ligninolytic laccase balances the process, minimizes the toxicity, and enables efficient heterologous secretion with a total protein yield of 13.7 g L-1. The secretory laccase complements the biochemical limits on lignin depolymerization well in *Rhodococcus opacus* PD630. Further proteomics analysis reveals the mechanisms for the oleaginous phenotype of *R. opacus* PD630, where a distinct multiunit fatty acid synthase I drives the carbon partition to storage lipid. The discovery guides the design of efficient lipid conversion from lignin and carbohydrate. The proteomics-guided integration of laccase-secretion and lipid production modules enables a high titer in converting lignin-enriched biorefinery waste to lipid. The fundamental mechanisms, engineering components, and design principle can empower transformative platforms for biomanufacturing and biorefining.

H&N LOQ Strawberry dietary fiber functionalized with phenolic antioxidants from olives. Interactions between polysaccharides and phenolic compounds

Bermúdez-Oria, A., *et al.*, *Food Chem. 280*: 310–320, 2019, https://doi.org/10.1016/j.foodchem.2018.12.057.

The interaction of strawberry cell wall with hydroxytyrosol (HT) and 3,4-dihydroxyphenylglycol (DHPG), two potent phenolic antioxidants naturally found in olive fruit with important biological properties, was investigated. The interaction occurred with drying and seemed to be more complex, strong, and irreversible than a simple association. MALDI TOF-TOF analysis suggested covalent (ester bond) and non-covalent (strong hydrogen-bonding, mostly) interactions. The oxygen radical absorbance capacity (ORAC) assay confirmed that the phenols partially maintained their antioxidant activity after binding to the soluble dietary fraction. This soluble dietary fiber was obtained following digestion simulated *in vitro* with gastric and intestinal fluids. Although the antioxidant activity of HT and DHPG was affected by the dietary fiber interaction, this activity was restored when polysaccharide size was reduced by enzymatic treatment, suggesting that a similar process could occur in the colon. Thus, the use of this novel antioxidant-enriched soluble dietary fiber as a functional food ingredient could potentially promote intestinal health.

H&N EAT LOQ Evaluation of juice and milk "food models" fortified with oxyresveratrol and beta-cyclodextrin

Matencio, A., *et al.*, *Food Hydrocolloids 98*: 105250, January 2020, https://doi.org/10.1016/j.foodhyd.2019.105250.

Despite being saturated, a huge quantity of "novel" functional food products reaches the food market every day, and companies are looking for new products to catch consumers' attention. In the present study, the inclusion complexes of oxyresveratrol with alpha-, beta-, and gamma-CD are characterized using DSC, TGA, SEM, and molecular docking, and their complexing capacities are compared. As beta-CD showed the best results, i) 0.2mM oxyresveratrol alone, ii) 0.2mM oxyresveratrol complexed with 8mM beta-CD and iii) 4mM oxyresveratrol solubilized with 8mM beta-CD were used to fortify juice and milk food models, which were kept in typical storage conditions (darkness and/or refrigerated) conditions for one month. The results showed that CD supplementation leads to a higher oxyresveratrol concentration and antioxidant capacity than when not used. Oxyresveratrol/beta-CD food models were stable for five weeks. The most typical variables measured were compared. *In vitro* digestion pointed to similar bioaccessibility. The bacteriostatic effect was also studied, and the greater effectiveness of oversaturated solutions was demonstrated. These results should be useful for the food industry for designing hydrophilic products containing oxyresveratrol.

H&N Parallel *in vitro* and *in silico* investigations into anti-inflammatory effects of non-prenylated stilbenoids

Leláková, V., *et al.*, *Food Chem. 285*: 431–440, 2019, https://doi.org/10.1016/j.foodchem.2019.01.128.

This study suggests that natural products can be repurposed to treat medical conditions with a similar efficacy as drugs. More work is warranted to bring natural products to market, as drug applications may help address the ever-increasing cost of primary health care.

Stilbenoids represent a large group of bioactive compounds which occur in food and medicinal plants. Twenty-five stilbenoids were screened *in vitro* for their ability to inhibit COX-1, COX-2 and 5-LOX. Piceatannol and pinostilbene showed activity comparable to the zileuton and ibuprofen, respectively. The anti-inflammatory potential of stilbenoids was further evaluated using THP-1 human monocytic leukemia cell line. Tests of the cytotoxicity on the THP-1 and HCT116 cell lines showed very low toxic effects. The tested stilbenoids were evaluated for their ability to attenuate

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the LPS-stimulated activation of NF-κB/AP-1. Most of the tested substances reduced the activity of NF-κB/AP-1 and later attenuated the expression of TNF-α. The effects of selected stilbenoids were further investigated on inflammatory signaling pathways. Non-prenylated stilbenoids regulated attenuation of NF-κB/AP-1 activity upstream by inhibiting the phosphorylation of MAPKs. A docking study used to in silico analyze the tested compounds confirmed their interaction with NF-κB, COX-2, and 5-LOX.

LOQ EAT A selective extraction of hydroxytyrosol-rich olive oil from alperujo

Tirado, D.F., *et al.*, *J. Food Eng. 263*: 409–416, 2019, https://doi.org/10.1016/j.jfoodeng.2019.07.030.

Alperujo, the solid-liquid waste generated by the current two-phase method of olive oil extraction, was dried, milled, and treated with supercritical carbon dioxide (sc-CO_2) to obtain a hydroxytyrosol (HT)-rich oil. At first, extraction rates were analyzed as a function of operating variables and the pre-condition of the raw material. Samples with particle size diameter <0.80mm and in equilibrium moisture (1%) with the atmosphere, improved oil extraction yield almost 40% compared with samples with the whole range of particle sizes. Extraction yield improved with solvent flow rate, but a minimum residence time was required. The optimum was 0.18 kg h–1 (7.5 kg CO_2 h–1 kg biomass–1). Higher pressures and lower temperatures resulted in higher extraction yields; at 30 MPa and 323 K the extraction curve slope was close to the theoretical oil solubility and the yield was 13%, like that obtained with n-hexane by Soxhlet (14%). However, the HPLC-DAD analysis identified higher HT concentration (1900 ppm) in the supercritical extracts at the highest temperature. Consequently, at 373 K, the total phenol content and the antioxidant capacity of the extracts was uppermost. No HT was found in the n-hexane extracts.

LOQ EAT The use of industrial thermal techniques to improve bioactive compound extraction and olive oil solid waste utilization

Lama-Muñoz, A., *et al.*, *Innov. Food Sci. Emerg. Technol. 55*: 11–17, 2019, https://doi.org/10.1016/j.ifset.2019.05.009.

The development of thermal treatments is helping to optimize the management of waste generated by the two-phase olive oil extraction system. A new treatment reactor, based on the steam explosion system, has been scaled up to the industrial level and offers several physical and chemical advantages. The reactor has been adapted to work into the pomace oil extractors. Chemical advantages include the efficient solubilization of simple phenols, like hydroxytyrosol, and sugars in the liquid fraction, and a significant reduction of humidity and toxicity of the residual solid fraction, in which cellulose and oil are concentrated, making the solid

residual waste more suitable for subsequent bioprocess applications—all of which will lead to the widespread use of steam treatment in the near future to improve the management and obtain the maximum exploitation of this byproduct.

LOQ EAT Effect of different microencapsulating materials and relative humidities on storage stability of microencapsulated grape pomace extract

Tolun, A., *et al.*, *Food Chem. 302*: 125347, January 2020, https://doi.org/10.1016/j.foodchem.2019.125347

This work aims to prolong the storage stability of polyphenols, obtained from grape pomace, using a spray drying-based microencapsulation technique. The microcapsules obtained under optimal conditions were stored at two different relative humidities (33% and 52%) during 75 days. The analyses of total phenolic content, antioxidant activity, and individual phenolic compounds were carried out every 15 days, and the most stable microcapsules were achieved with maltodextrin DE_{4-7} prepared by adding gum Arabic to the wall material at a ratio of 8:2. The phenolic content loss rate was found to be in a range of 0.93–5.42 % depending on phenolic compound. The decrease in the content of rutin, chlorogenic acid, epicatechin, caffeic acid, gallic acid, caftaric acid, and catechin was only 0.93, 2.09, 2.13, 2.27, 2.41, 3.40, and 5.42%, respectively. These results indicate more efficient storage conditions than those of previously reported studies.

PRO Optimization of ultrasound-assisted ethanol extraction of hazelnut oil

Wong, S.-T., *et al.*, *J. Food Process. Pres.*, online first August 2019, https://doi.org/10.1111/jfpp.14138.

In this study, ethanol was used as a polar solvent to extract hazelnut oil, with ultrasound aid in the first 15 min of extraction. A Box–Behnken design was used for optimization in terms of ultrasound amplitude (30%, 60%, and 90%), extraction temperature (28, 38, and 48°C), and extraction time (30, 60, and 90 min). All three factors showed positive effects on the oil extraction. The results showed that the oil yield and quality of extracted oil were significantly affected by the application of ultrasound. Ultrasound treatment had increased the oil yield from 38.93% up to 79.88%. The optimum conditions were identified at 90% of ultrasound amplitude, 29°C of extraction temperature, and 51 min of extraction time. Under these conditions, the predicted maximum oil yield was 55.39% with the minimum FFA value, iodine value and peroxide value of 1.75%, 14.52 g/100 g, and 10.50 meq g O_2/kg, respectively.

PRO Renewable resources from insects: exploitation, properties, and refining of fat obtained by cold-pressing from *Hermetia illucens* (black soldier fly) larvae

Matthäus, B., *et al.*, *Eur. J. Lipid Sci. Technol. 121*: 1800376, 2019, https://doi.org/10.1002/ejlt.201800376.

Hermetia illucens (black soldier fly) larvae are dried and mechanically treated by an oil seed screw press to extract the fat. This leads to an accumulation of the protein in the press cake with a protein content of 42% dry base. The amino acid composition fulfills the scoring patterns of FAO/WHO for adults. The extracted fat fraction has an interesting fatty acid composition with lauric acid (48%), myristic acid (11%), and palmitic acid (16%) as predominant representatives, very similar to palm kernel fat and coconut fat. The content of tocopherols and tocotrienols is low (64.7 mg kg^{-1}) while the total amount of sterols (3557 mg kg^{-1}) is comparable or higher to commonly used vegetable fats and oils. The sterol composition is dominated by campesterol (889.7 mg kg^{-1}) and beta-sitosterol (1866 mg kg^{-1}). The high content of delta5-avenasterol may result in a higher thermal stability during food processing. The triacylglycerol composition of the fat is determined by LaLaLa (27.6%), LaLaM (16.0%), and LaMM (15.1%) resulting in DSC thermograms very similar to palm kernel and coconut fat with comparable exothermic (8.98 and 3.57°C) and endothermic (27.23°C) peaks. This gives the oil a melting and crystallization behavior comparable to that of palm

kernel fat and could therefore be used in similar food applications. Since insect fat is only consumable after refining, the current work also investigates the effect of refining on some physical and chemical parameters. After refining the quality of the fat is within the limits defined by the Codex Alimentarius for Named Vegetable Oils and Fats.

PRO Aqueous-phase glycerol catalysis and kinetics with *in situ* hydrogen formation

Torres, A., *et. al.*, *ACS Sustain. Chem. Eng.* 7: 11323–11333, 2019, https://doi.org/10.1021/acssuschemeng.9b00807.

Hydrodeoxygenation (HDO) is a widely used technology to convert biomass-based feedstock into value-added fuels and chemical products that usually require high pressure hydrogen (H_2) to remove excess oxygen in the biomass feedstock. To make this process safer and more sustainable, we investigated glycerol conversion under an inert atmosphere in aqueous media using multimetallic catalysts (comprised of Ru, Re, and Pt) supported on activated carbon. Here we report a trimetallic Ru-Re-Pt catalyst that converts glycerol to value-added products such as 1,2-propanediol (1,2-PDO) and linear alcohols with higher selectivity with *in situ* formed H_2 (without using external H_2). Thus, the proposed system eliminates use of expensive hydrogen while giving high selectivity unlike the hydrogenation with external hydrogen. The results of catalyst screening showed high glycerol conversion (83%) with liquid-phase product selectivity of 72% and 1,2-PDO selectivity of 43% at 493 K and autogenous pressure with optimized Ru-Re-Pt/C trimetallic catalyst in the presence of a solid base promoter (MgO). To infer the mechanism of hydrogenation with *in situ*-formed H_2, temporal concentration-time profiles at various operating conditions were elucidated using a kinetic model. Such a model provides valuable mechanistic insights and guidance for developing optimal catalyst formulations for maximizing hydrodeoxygenation products with *in situ*-formed hydrogen.

PRO S&D BIO Integrated production and separation of biosurfactants

Dolman, B.M., *Process Biochem.* 83: 1–8, 2019, https://doi.org/10.1016/j.procbio.2019.05.002.

Environmentally friendly, microbially produced surfactants known as biosurfactants have recently seen an explosion in commercial activity and interest due to a reduction in the cost of production, though these costs still limit biosurfactant use in bulk applications. These high production costs are primarily the result of the typically low productivities of large-scale biosurfactant production processes and hence the large production volumes required, as well as process engineering challenges related to the nature of the biosurfactant produced. This review details the use of integrated separation technologies, primarily gravity, membrane, and foam fractionation separations, in integrated biosurfactant producing fermentations, to tackle these difficulties. An analysis of the scalability of the available technologies and the expected impact on process economics is presented, demonstrating the potential utility

of integrated separation processes for bringing biosurfactants into mainstream commercialization.

S&D ANA Imaging of compositional gradients during *in situ* emulsification using X-ray micro-tomography

Unsal, E., *et al. J. Colloid Interf. Sci. 550*: 159–169, 2019, https://doi.org/10.1016/j.jcis.2019.04.068.

The rate of emulsification in surfactant/oil/water systems is influenced by transport of chemicals and mixing of the fluid phases. In porous media applications, complex flow regimes are generated due to three-dimensional connectivity and irregular cross-sections of the pores facilitating the mixing for emulsification. The properties of the resulting emulsified phase depend on the interplay of flow, mixing, and emulsification kinetics of the surfactant/oil/water system. Emulsification can be relatively quick. Direct visualization of the process and compositional gradients in three-dimensional pore space during flow requires imaging at few seconds time intervals. In this study, a flow unit was integrated in a synchrotron beamline-based fast X-ray computed micro-tomography set-up. Non-destructive three-dimensional visualization of multi-phase flow inside a porous rock at flow conditions became viable. An oil saturated rock sample was first flooded with water, followed by surfactant solution to mobilize the remaining oil by miscible displacement. The sample was continuously imaged during injection; the scans were made at time intervals of 7–6 s. The presence of an emulsified phase in addition to the oil and the aqueous phases required a more advanced image processing workflow compared to the workflows used for the immiscible fluid systems. A newly developed image processing technique was adopted; the grey levels in the images were correlated with the local oil content in the emulsified fluid regions. The visual extractions of the pore space showed that the emulsification occurred within seconds. Compositional gradients were observed in the emulsified phase as the injected surfactant solution reached the remote locations in the pore space. While a significant fraction of the oil was displaced within few seconds, the compositional gradients persisted over several millimeter length for several minutes, illustrating a sequence of mobilization and solubilization of the oil phase. The ability to interpret such compositional gradients in real time in porous space brings capability to study interfacial phenomena in applications where in situ emulsification occurs under flow.

S&D ANA Electrophoretic NMR characterization of charged side chain cationic polyelectrolytes and their interaction with the anionic surfactant, sodium dodecyl sulfate

Patel, L., *et. al.*, Langmuir 35: 9233–9238, 2019, https://doi.org/10.1021/acs.langmuir.9b01324.

Oppositely charged polymers and surfactants show a complex phase behavior with large regions of solubility and insolubility dependent on the concentrations of the species present. Here, a series of quaternized hydroxyethyl cellulose (cationic) polymers have been characterized by pulsed-gradient spin-echo NMR (PGSE-NMR) and electrophoretic NMR (eNMR) in simple aqueous (D2O) solutions and in combination with the oppositely charged (anionic) surfactant, sodium dodecyl sulfate (SDS). Analysis of the effective charge on the polymer derived from both the eNMR and PGSE-NMR results yields a readily interpretable insight into the polymer behavior; the effective charge on the polymer at infinite dilution shows a linear relationship with the degree of modification. On addition of low concentrations of SDS, typically C-surf < 5 mM, the surfactant interacts with the charged polymers, leading to substantial changes in the dynamics of the system (polymer diffusion, viscosity). At these levels of surfactant addition, there is no macroscopic phase separation. Further, with the absence of an interaction with the parent, the uncharged polymer strongly suggests that the SDS only interacts with the charged moieties present on the functionalized side groups and not the polymer backbone. Ultimately, the charge on the soluble polymer/surfactant complex was found to depend linearly on the level of surfactant binding across a series of polymers with differing levels of modification with the charge becoming effectively zero at the macroscopic phase separation boundary.

S&D ANA From well-entangled to partially entangled wormlike micelles

Zou, W., *et. al.*, *Soft Matter 15*: 642–655, 2019, https://doi.org/10.1039/c8sm02223b.

We combine mechanical rheometry, DWS, and SANS with a simulation model, the "pointer algorithm," to obtain character-

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PS Form **3526,** July 2014 *[Page 1 of 4 (see instructions page 4)]* PSN: 7530-01-000-9931 **PRIVACY NOTICE:** See our privacy policy on *www.usps.com.*

13. Publication Title: INFORM			14. Issue Date for Circulation Data Below	
15. **Extent and Nature of Circulation**			**Average No. Copies Each Issue During Preceding 12 Months**	**No. Copies of Single Issue Published Nearest to Filing Date**
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b. Paid Circulation *(By Mail and Outside the Mail)*	(1)	Mailed Outside-County Paid Subscriptions Stated on PS Form 3541 (Include paid distribution above nominal rate, advertiser's proof copies, and exchange copies)	2443	2570
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f. Total Distribution *(Sum of 15c and 15e)* ▶			2463	2712
g. Copies not Distributed *(See Instructions to Publishers #4 (page #3))* ▶			80	63
h. Total *(Sum of 15f and g)*			2543	2775
i. Percent Paid *(15c divided by 15f times 100)* ▶			91.74	98.08

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18. Signature and Title of Editor, Publisher, Business Manager, or Owner: Gloria B Cook, Sr. Director, Finance & Operations — Date: August 7, 2019

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istic lengths and time constants for WLM solutions over a range of salt concentrations encompassing the transition from unentangled to entangled solutions. The solutions contain sodium lauryl ethylene glycol sulfate (SLE1S), cocamidopropyl betaine (CAPB), and NaCl. The pointer algorithm is extended to include relaxation of unentangled micelles, allowing micelle parameters to be extracted from the rheology of partially entangled solutions. DWS provides the data at high frequency needed to determine micelle persistence length accurately. From pointer algorithm fits to rheology, we observe a salt-induced rapid change in micellar length as the solution enters the well-entangled regime and a weaker growth with surfactant concentration consistent with mean-field theory. At a lower surfactant concentration, micelle length and persistence length from SANS are roughly consistent with values from rheology once the lower surfactant concentration used in SANS is accounted for. This is, to our knowledge, the first time that quantitative comparisons of structural features including micelle length are made between rheology and SANS. Finally, scaling laws for micelle diffusion and recombination times indicate that micelle kinetics are reaction controlled leading to mean-field recombination with surrounding micelles over the entire range of concentration of interest except at very low and very high surfactant concentrations where either short micelles or branched micelle clusters are dominant.

S&D ANA Systematically quantifying oil-water microemulsion structures using (spin-echo) small angle neutron scattering

Mulder, M., *et al. Colloids Surf. A 575*: 166–175, 2019, https://doi.org/10.1016/j.colsurfa.2019.04.045.

Microemulsion systems consisting of D_2O, an alkane, an anionic internal olefin sulfonate surfactant, salt, and secondary butyl alcohol (SBA) as co-solvent are studied in a systematic way. In four different sample sets, either the salt content, SBA content, or alkane carbon number was varied to study the effects of the individual compounds on the structure sizes making up the microemulsion. Using complementary small-angle neutron scattering techniques SANS and Spin-Echo SANS, it was found that the microemulsion systems exhibit the largest structures in the optimum state (domain size of d/2 =144 nm in the model by Teubner and Strey), where the structure is considered bicontinuous. In comparison, at under- and over-optimum states where the structures consist of emulsified spherical droplets, the smallest measured diameter was 2R = 44 nm. Furthermore, the structure sizes in bicontinuous microemulsions decrease exponentially (down to d/2 =15 nm for pentadecane and 5 wt% SBA) as function of both SBA content and alkane carbon number. The observed trends in structure sizes combined with the trends observed in the area per surfactant molecule, are qualitatively explained with the extended Winsor R-ratio, the HLD-NAC model, and surfactant film flexibility arguments.

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