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Trends in synthetic and natural antioxidants for shelf life extension of meat and meat products

F.S.H. Lu and A. Pham-Mondala

- Naturally sourced antioxidants have gained considerable attention in the food industry due to consumers' preference for natural ingredients.
- An optimal combination of naturally sourced ingredients is important to retard spoilage and quality deterioration of meat from lipid oxidation, microbial growth, and other mechanisms.
- Our studies suggest that it is feasible to use naturally sourced antioxidants to maintain color and flavor stability, and subsequently to extend the shelf life of fresh, pre-cooked, cooked, and processed meat.

In meat, the presence of unsaturated fat in membrane phospholipids causes fat to oxidize during processing and storage. As a result, the quality of meat and meat products deteriorate when fat oxidizes and develops off-flavors. Lipid oxidation increases the conversion rate of oxymyoglobin (bright red color) to metmyoglobin (brown discoloration) and subsequently impacts the physical appearance of meat and meat products [1]. Meat is also very susceptible to spoilage and microbial growth during slaughtering and post-slaughter handling. Therefore, meat suppliers use various food additives to extend the shelf life of meat and meat products. These typically include synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallate, and TBHQ (tert-butylhydroquinone). Nevertheless, due to increasing demands for clean label solutions, extensive work has been conducted to identify novel and natural extracts with potential applications in meat and meat products.

Studies using natural extracts with potential applications in meat and meat products were reviewed by Kumar and colleagues. Examples of the natural extracts, active ingredients, and applications that have been studied can be seen in Table 1. [2].

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TABLE 1. Antioxidants from natural sources with potential applications in meat and meat products

Natural sources	Active ingredients	Applications
Oregano essential oil	Thymol, para-cymene, gamma-terpinene, carvacrol	Raw and cooked porcine/bovine meat
Sage essential oil	Eucalyptol, camphor, alpha-pinene	Raw and cooked porcine/bovine meat
Curry and mint leaves	phenolics	Raw ground pork meat
Rosemary and oregano leaves extracts	Phenolics, rosmarinic acid, carnosic acid, carnosol	Raw pork batter
Grape seeds and peels extract	phenolics	Raw and cooked ground chicken
Green tea extract	polyphenols	Sucuk
Borage leaves extract	phenolics	Dry fermented sausage enriched with omega-3 polyunsaturated fatty acids
Sea buckthorn (berry residues)	polyphenols	Raw and cooked mechanically deboned chicken and turkey
Defatted canola meal	Sinapic, ferulic, para-hydrobenzoic acid	Chicken meat homogenate
Red grape pomace extract	Total polyphenolics, total anthocyanins	Pork burger

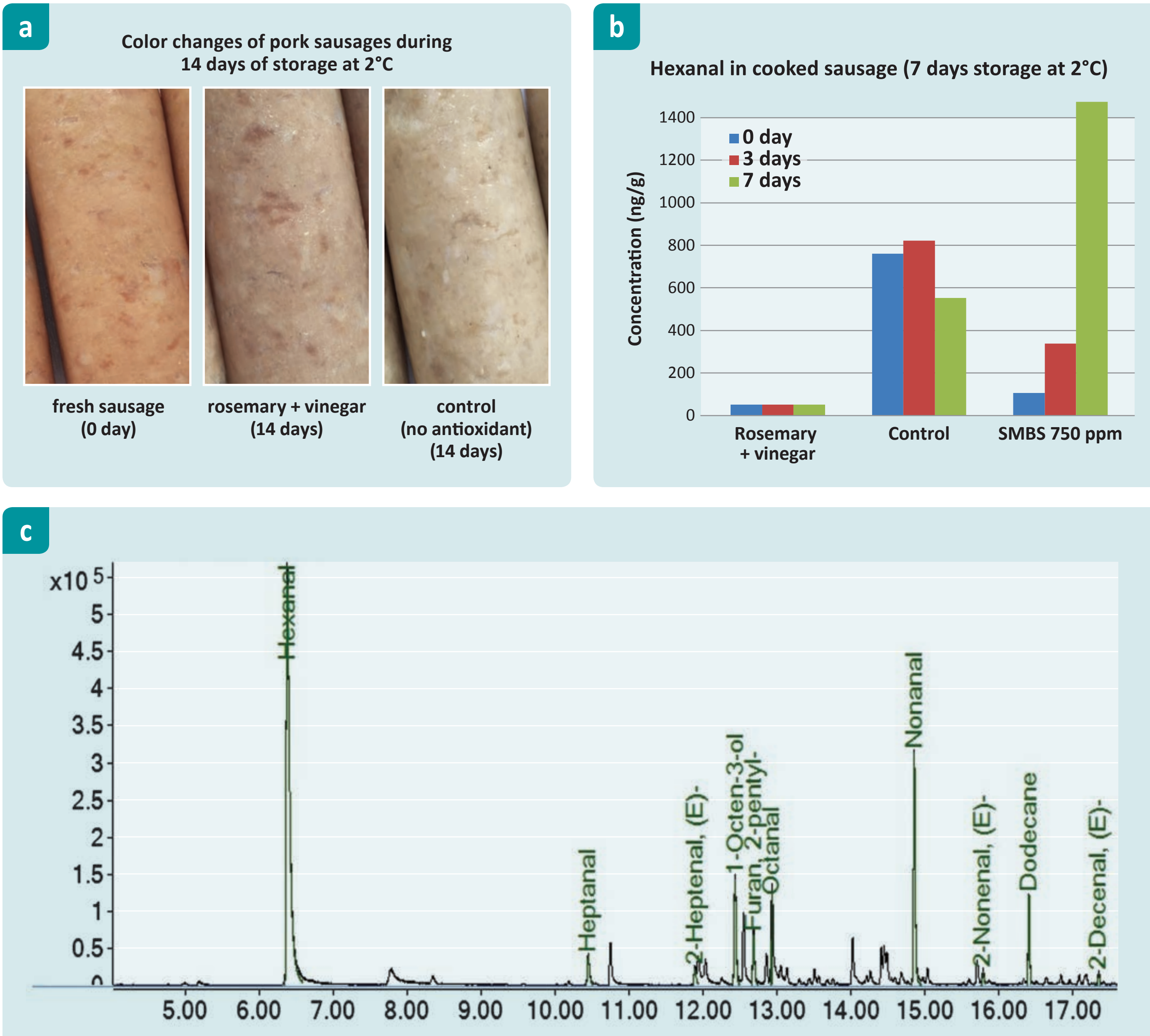

FIG. 1. a) Color changes of pork sausages during 14 days of storage at 2°C; b) the hexanal level in cooked sausages after 7 days of storage at 2°C; c) the profile of secondary volatile oxidation products detected in oxidized pork sausage

The most commonly used naturally sourced antioxidants are phenolic compounds such as phenolic acids, tocopherol, and flavonoids. Phenolics prevent lipid oxidation through different mechanisms—by functioning either as free radical scavengers, metal chelators, or singlet oxygen quenchers. The antioxidative potential of phenolics depends on their skeleton structure. The number and location of functional groups, such as free hydroxyl OH groups, is just one example. For instance, phenolics with a higher number of OH groups and ortho-3,4-dihydroxy structures will have higher antioxidative properties [3]. In addition, some phenolics, such as carnosic acid from rosemary, and catechin from green tea, not only have OH groups that can donate hydrogen to free radicals, but also contain vicinal -OH groups that can chelate metals. Consequently, combinations of natural extracts can potentially deliver synergistic effects and improve antioxidant performance in preventing lipid oxidation. Such combinations can also reduce the effective dosage of each extract, thus minimizing impacts on flavor and color.

Fresh meat, including sausages, are a major category of meat retail sales, both in the United States and Europe. Fresh sausages contain a high level of fat and are prepared from fresh comminuted meat from different meat types such as pork, chicken, beef, and so on. Therefore, fresh sausages are highly perishable, especially when these products are refrigerated (2–5°C) in oxygen semi-permeable packaging. In some European countries, such as the United Kingdom, Australia, and New Zealand, sulfur dioxide (mainly in the form of Sodium metabisulphite, SMBS) is a commonly used antimicrobial agent and color preserva-

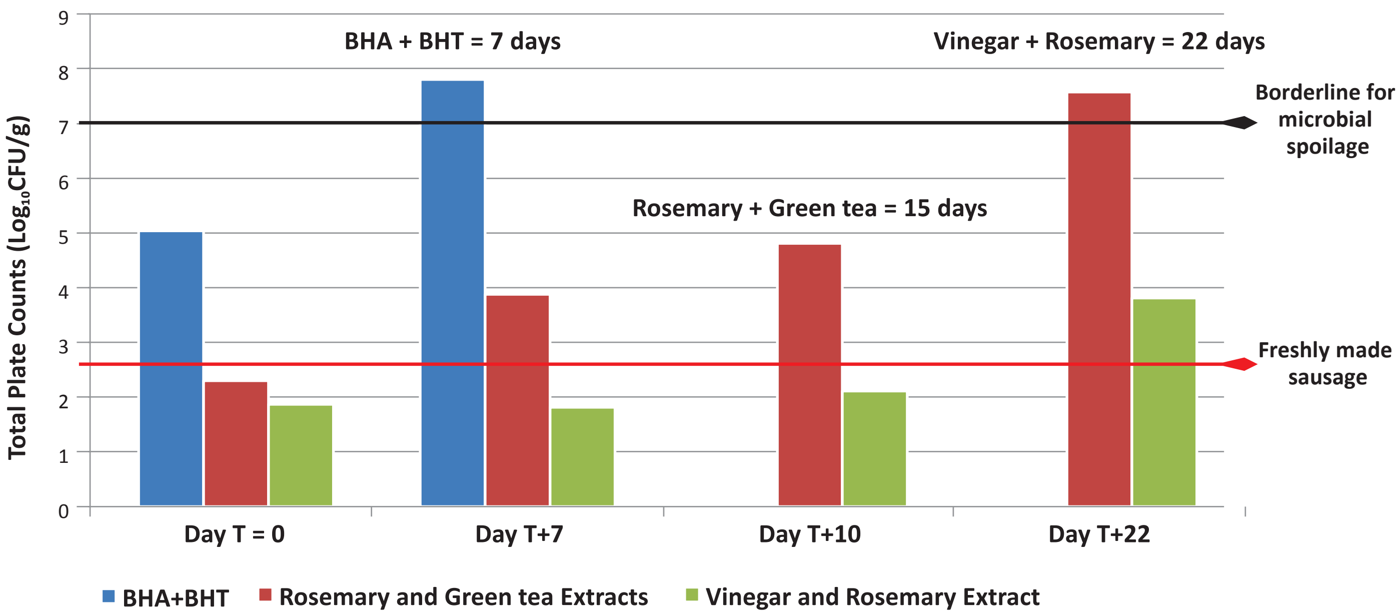

FIG. 2. Total plate count of US pork sausages treated with a) BHA and BHT; b) rosemary and green tea extracts, c) vinegar and rosemary extracts during real-time shelf life study at 3°C for 22 days

tive used to maintain the quality of fresh sausages, with the maximum permissible limit in Europe of 500 mg/kg of sulfur dioxide/sulfites. The use of SMBS in meat is not allowed in the United States, as it causes a significant loss of thiamin. Consequently, synthetic antioxidants such as BHA, BHT, and propyl gallate are commonly used to extend the shelf life of fresh sausages in the US market. Three main mechanisms responsible for the spoilage of sausages are lipid oxidation, microbial growth, and enzymatic autolysis (such as proteolysis and lipolysis). Of these, microbial growth is the main cause of spoilage for sausages [4, 5].

For this reason, naturally sourced ingredients comprising antioxidative and antimicrobial agents were developed in our laboratory to delay the spoilage of pork sausages from different mechanisms, and to meet the different needs of US, UK, and EU markets. A storage study was conducted to investigate the efficacy of a natural blend comprising rosemary extract and buffered vinegar to extend the shelf life of British pork sausages. British pork sausages (70% minced pork, 15% water, 13% rusk and flour) were treated either with 0.075% of SMBS or 1.0% of the natural additives. The sausages were stored at 2°C under polyvinyl chloride (PVC) overwrap packaging for 14 days. The obtained results showed that SMBS is only effective in stabilizing the color of pork sausages for the first few days, whereas naturally sourced ingredients were superior in maintaining the color of pork sausages even after 14 days of storage (Fig. 1a). In addition, these naturally sourced ingredients provided a performance comparable to that of SMBS in inhibiting the growth of spoilage bacteria (total plate count of 3.6 log $_{10}$ CFU/g versus 2.9 log $_{10}$ CFU/g quantified at 7 days of storage), yet had the lowest lipid oxidation level (as shown by the hexanal level in Fig. 1b). The degree of lipid oxidation in sausages was monitored through the hexanal level, and using other selected oxidation markers such as heptanal, 1-octen-3-ol, and nonanal (Fig. 1c).

Similarly, a study was conducted to investigate the efficacy of natural antioxidant combinations (0.5% rosemary extract combined with buffered vinegar, and 0.2% rosemary extract combined with green tea) in prolonging the shelf life of US pork sausage relative to that of synthetic antioxidants. (0.02% BHA and BHT based on fat content). To mimic sausages in the US supply chain, pork sausages (90% minced pork, 3% water) were frozen at -20°C for 3 months, followed by refrigerated storage at 3°C for 22 days. Judging from the data of total plate count (Fig. 2), the combination of rosemary extract and buffered vinegar provided the longest shelf life to pork sausages (more than 22 days), followed by the combination of rosemary extract and green tea (15 days) and the combination of BHT and BHA, which showed the shortest shelf

FIG. 3. Visual observation of ground beef packed under high oxygen MAP condition at 3–4°C for 12 days (away from light) without antioxidant (left) and with rosemary extract (right)

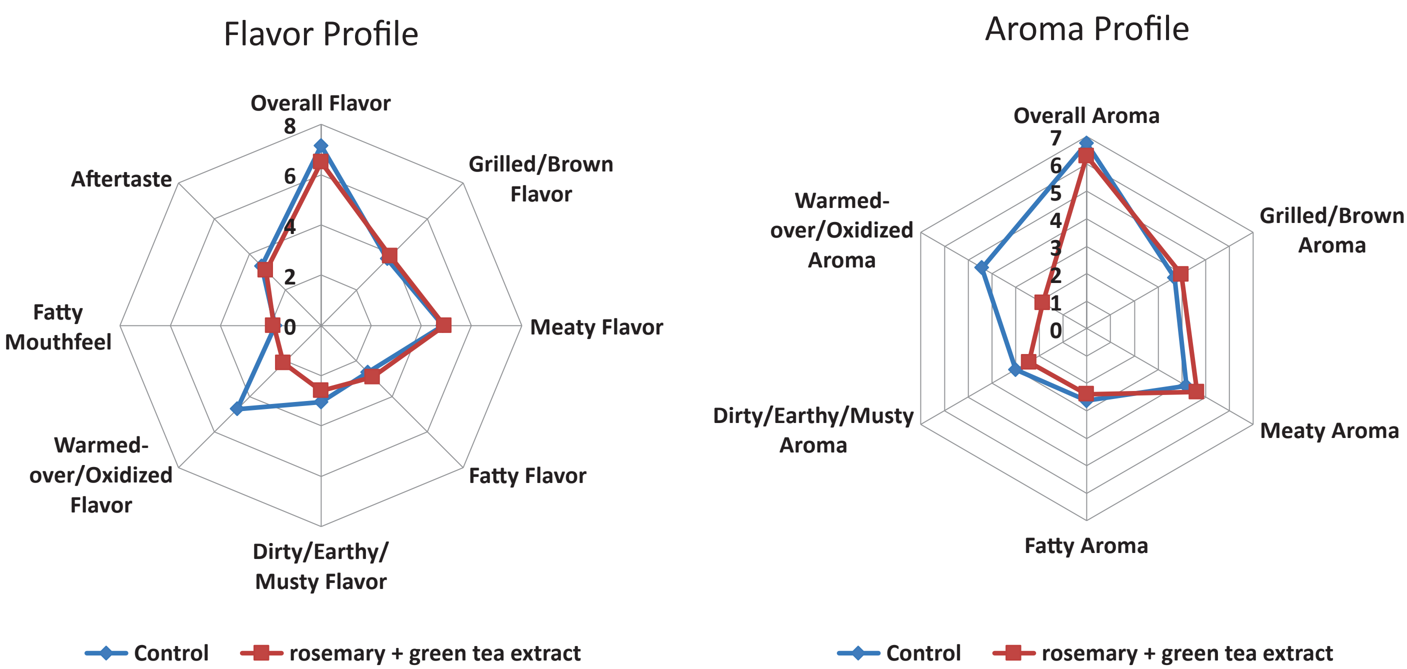

FIG. 4. Sensory profile of 95% lean cooked beef stored at 4°C for 3 days' storage under PVC overwrap (obtained from 20 trained panelists)

life (7 days). Our studies showed that a natural combination of antioxidants performed better than synthetic antioxidants in extending the shelf life of fresh pork sausages.

As consumers typically associate the freshness of meat with redness, they may reject meat and meat products that are discolored. In the meat industry, modified atmosphere packaging (MAP) has been used extensively to preserve meat color. Our internal studies have proven that incorporation of rosemary extract alone could further improve the color and flavor stability of fresh ground beef packed under MAP condition (Fig. 3). Our studies also proved that the use of antioxidant combinations (rosemary and acerola extracts) could provide additional color and flavor stability to fresh ground beef compared to rosemary extract alone.

In contrast with raw meat, undesirable off-flavors and aromas in cooked meat are often described as having a "warmed over flavor" (WOF), and different antioxidants are required to delay the development of these off-notes in cooked meat systems. Trials were conducted in our laboratory to investigate the efficiency of a combination of antioxidants (rosemary and green tea extract) to reduce WOF flavor and aroma in cooked beef. Our findings showed that the flavor stability of cooked beef treated with rosemary and green tea extracts was improved as evidenced by a lower level of WOF flavor and aroma (Fig. 4). The details of the above-mentioned studies have been published by Pham-Mondala and colleagues elsewhere [6], and therefore are not further discussed in this article.

As with raw and cooked meat, there is an increasing interest in using naturally sourced antioxidants to maintain the color and flavor stability of processed meat, such as dry fermented sausage. In the United States, dry fermented sausage is usually formulated to contain 45% meat block and 42% fat in the finished product, with a moisture and protein ratio of 1.6:1, a pH less than 5, and a salt content of 4% by weight. Due to its high fat content, dry fermented sausage is highly susceptible to oxidation—especially when it is sliced and not stored under a protective atmosphere. Typically, nitrites in the form of sodium nitrite or potassium are used by the meat industry to stabilize cured meat flavor and color, and to limit the growth of anaerobic bacteria and other microbes [5, 7]. The current permissible level of nitrites in meat products is 156 ppm in the United States and 200 ppm in Canada. To provide an alternative to conventional curing using nitrites, our laboratory has evaluated the efficiency of using a natural cure (150 ppm sodium nitrite from pre-converted vegetable juice powder, 0.12% of acerola powder, rosemary, and green tea extracts) versus conventional cure (150 ppm sodium nitrite, 400 ppm ascorbic acid, 60 ppm BHA/BHT) in maintaining the quality and shelf-life of dry-fermented sausage. In this study, the sausages were packed under high oxygen MAP condition (80% O_2 and 20% CO_2) to accelerate oxidation. Storage was conducted at a refrigerated temperature of 3°C for 28 days. The obtained results showed that a natural cure with rosemary and green tea extracts was as effective as a conventional cure in reducing the oxidation level in the sausages, but with a better maintenance of color (changes of a* value, Fig. 5, page 12). Our studies demonstrated that it is feasible to use a natural cure in extending the shelf life of dry fermented sausages.

In conclusion, our internal studies demonstrated that it is feasible to use naturally sourced food ingredients to maintain color, retard the development of off-notes, and limit microbial growth in meat and meat products. We also learned that

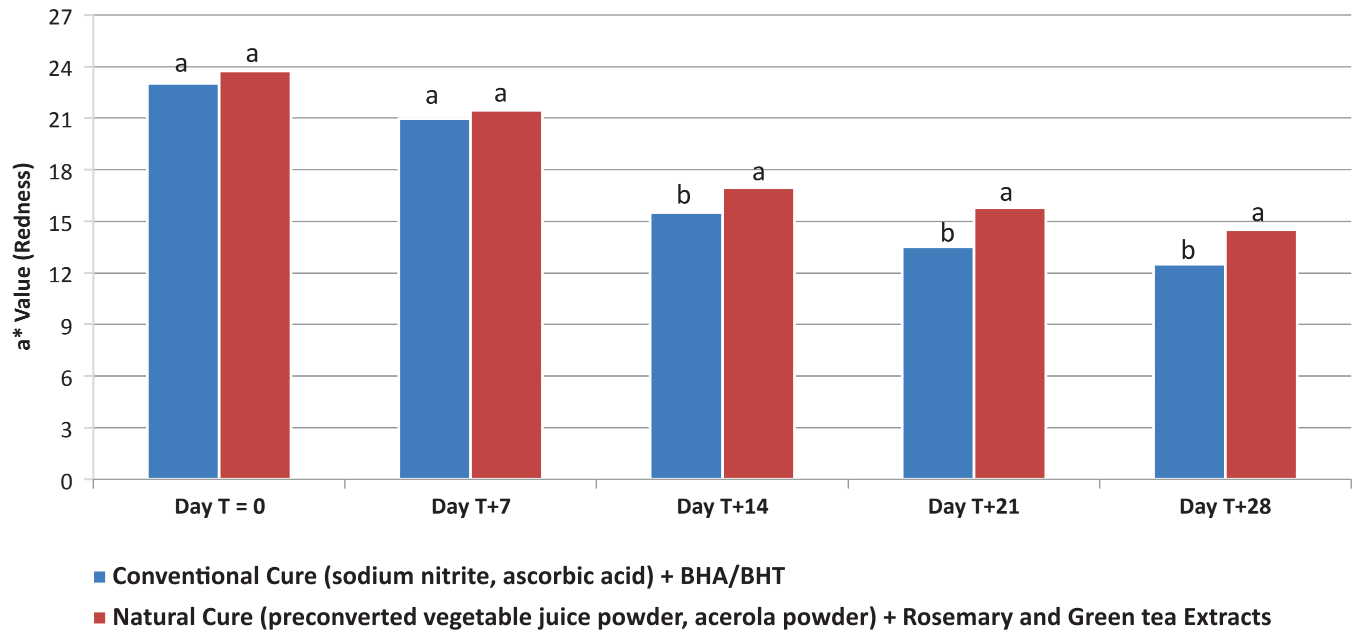

FIG. 5. The changes of a* values (redness) of dry fermented sausage stored under high oxygen MAP packaging (80 O_2 and 20 CO_2) at 3±1°C for 28 days

combinations of naturally sourced food ingredients are more effective at extending the shelf life of complex meat systems, as they delay spoilage from different causes.

F.S.H Lu received her PhD in Food Science from the Technical University of Denmark, where she investigated the oxidative stability of omega-3 for food applications and won an AOCS honored student award for her widely published research. In 2014, Lu joined Mondelez UK as a lipid scientist, expanding her knowledge in analytical techniques for studying oxidative stability, antioxidants, and shelf life prediction. In 2017, she joined Kalsec Europe Ltd as a lead scientist, serving as a principle investigator for projects involving the use of antioxidants. Lu supports research in the areas of lipid oxidation, antioxidants, and shelf life extension of meat products for the European market. She can be contacted at hlu@kalsec.com.

A. Pham-Mondala earned Ph.D. and MS degrees in Food Science, Nutrition, and Health Promotion from Mississippi State University. Her research focused on developing antioxidant combinations to extend the shelf life and quality of fresh meat products during processing and storage. In 2016, Pham-Mondala joined Kalsec Inc. as a senior scientist in Meat and Poultry Applications/Antioxidants. In this role, she supports the Meat, Poultry, and Pet Food Team on product development for meat and poultry applications, conducts research for developing all-natural innovative products and solutions in key food applications, and real-time analytical support to meet industry needs. She can be contacted at apham@kalsec.com.

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Oilseeds in Sudan: poised for growth

Catherine Watkins

- After years of political turmoil and economic sanctions, Sudan appears to be poised for development.
- The lifting in late 2017 of most economic sanctions placed on the country by the United States has opened the Sudan's oilseed industry to foreign investment.
- Although oilseed yields are quite variable and suffered a period of decline, signs point to an upswing in production.

An analysis of oilseed production and oil processing in Sudan requires both a broad and a long view. The history of the country—first as what was essentially a British colony with a governor-general appointed by Egypt, and then post-independence from 1956 on—has shaped the present. The toll of political upheaval; economic sanctions (levied in 1997 by the United States, expanded in 2006, and mainly lifted in October 2017); and the formal separation of The Republic of Sudan and South Sudan in 2011 all play a role in why Sudan is poised now for investment and development. Talk to those with knowledge of the country, and the same words continually arise: "potential" and "opportunity."

A BRIEF HISTORY

Sudan, which sprawls in northeastern Africa directly below Egypt, was the largest country in Africa prior to the secession of South Sudan in 2011, which took with it close to 27% of the combined country's landmass, more than 50% of its oil revenue, and 95% of its exports. After the secession of South Sudan, the Republic of Sudan became the fifteenth-largest country in the world in terms of land area—with almost 1.9 million square kilometers (about 735,000 square miles)—of which about 30% is agricultural land. There are 195 countries in the world.

"Agriculture—including crops, livestock, forests, fish, and wildlife—has been and will continue to be for some time the backbone and engine of the national economy," said Mohamed Elwathig Saeed Mirghani in an email. Mirghani is an associate professor in the Department of Biotechnology Engineering at International Islamic University Malaysia (IIUM) in Kuala Lumpur. He is also a co-founder of the National Oilseed Processing Research Institute (NOPRI) at the University of Gezira in Khartoum (see sidebar).

TABLE 1. Quantity and value of Sudan's oilseed exports

Sudan Oilseeds Export Quantity (tonnes)

Item	Year	Value
Groundnuts, shelled	2012	109
Groundnuts, shelled	2013	5,251
Oil, groundnut	2012	3
Oil, groundnut	2013	2,428
Oil, sesame	2012	144
Oil, sesame	2013	181
Oil, sunflower	2012	22
Oil, sunflower	2013	0
Sesame seed	2012	175,476
Sesame seed	2013	219,635

FAOSTAT, 2018

Sudan Oilseeds Export Value (1000 US$)

Item	Year	Value
Groundnuts, shelled	2012	79
Groundnuts, shelled	2013	4,901
Oil, groundnut	2012	0
Sesame seed	2012	187,172
Sesame seed	2013	346,413
Oil, groundnut	2013	3,600
Oil, sesame	2012	197
Oil, sesame	2013	280
Oil, sunflower	2012	42
Oil, sunflower	2013	0

FAOSTAT, 2018

Sudan has several important production regions for oilseeds, according to Elfadl Yousif Elmogtaba Elfadl, an assistant professor at NOPRI, who spoke in an email about the country's "huge water and land resources located in a number of climatic zones." Indeed, production of the three main oilseed crops—groundnuts (peanuts), sesame, and sunflower—as well as cottonseed, castor bean, soybeans, melon seed (*Citrullus colocynthis*), and other niche oils occurs under both irrigated and rain-fed conditions.

Yields, however, are highly variable and are only now—with the exception of cottonseed—stabilizing after a downward trend that began in the mid-1980s (see Tables 1 and 2).

In general, productivity tends to be higher in crops grown under irrigation than those grown in rain-fed areas. Two reasons given for the loss of productivity, particularly in rain-fed areas, are the natural resource degradation from the expansion of cultivated area into marginal lands as well as soil depletion from monocropping practices. Nonetheless, all sources for this article agree there is great potential for Sudan to diversify its economy and bring the days of higher yields back.

Nearly all the vegetable oil that is produced is consumed by the domestic market, either as edible oil or for use in cosmetics, medicine, soap manufacturing, flavoring, and perfumes. Overall, however, Sudan is a net exporter of oilseeds. In fact, as much as 40% of Sudan's sesame seed production is exported to top importers that include Egypt, Republic of Korea, Saudi Arabia, Syria, Lebanon, and China. Cottonseed is crushed domestically to produce cooking oil, and cotton cake is an important feed product.

PALM OIL IN SUDAN

Sudan has not yet developed a palm oil industry. Oil palm (*Elaeis guineensis*) has its origins in West Africa, and there is evidence of the consumption of palm oil in ancient Egypt. So why hasn't an oil palm industry flourished in Sudan?

Oil palm was introduced to the country in 1900 by the British, explained Ismail Hassan Hussein Abdalnabi, a professor at NOPRI and long-time AOCS member. "In 1993, we suggested that the federal Ministry of Agriculture and Forestry add oil palm to its list of promising crops; a national team was set up, and NOPRI undertook the task of supplying seedlings from Malaysia," he added. Seedlings were test-grown in 10 states; Abu Karshola in the state of South Kordofan was the most promising site.

To establish oil palm production will require government spending before the private sector can be called upon to invest, Abdalnabi suggested. Because oil palm trees take 27 to 48 months to mature and require nurturing for their productive life of as much as 25 to 35 years, "such projects must be part of an overall governmental strategy," he noted, "which may explain why the oil palm sector succeeded in Malaysia but was not that fortunate elsewhere."

OILSEED PROCESSING IN SUDAN

The edible oil industry in Sudan has a long precolonial history, said IIUM's Mohamed Mirghani. (Britain, for all intents and purposes, ruled Sudan from 1899 to 1956.) For many hundreds

TABLE 2. Oilseed production in Sudan 1969–2018 (Metric tons)

	Cottonseed		Peanut	
Years	Oil	Oilseed	Oil	Oilseed
1969	63	467		
1970	60	465		
1971	63	454		
1972	50	358	29	568
1973	63	432	72	544
1974	61	416	90	930
1975	31	204	98	931
1976	44	297	105	705
1977	51	348	126	1,027
1978	38	261	163	815
1979	33	228	175	852
1980	30	207	121	707
1981	48	332	136	740
1982	64	441	69	497
1983	70	475	59	413
1984	65	434	55	390
1985	44	304	38	275
1986	51	349	61	380
1987	42	291	58	435
1988	43	297	58	450
1989	39	272	64	400
1990	27	191	47	325
1991	27	190	64	400
1992	16	150	64	390
1993	16	109	64	390
1994	29	201	64	390
1995	32	249	58	370
1996	34	249	58	370
1997	31	249	64	370
1998	18	107	66	370
1999	17	123	67	370
2000	23	174	165	947
2001	23	169	160	990
2002	26	190	165	1,267
2003	23	159	150	790
2004	38	269	105	790
2005	23	169	68	520
2006	19	138	80	555
2007	6	54	80	564
2008	9	69	110	716
2009	3	24	155	942
2010	3	27	150	763
2011	10	101	150	1,185
2012	4	37	150	1,032
2013	9	83	190	1,767
2014	8	72	190	1,871
2015	12	108	220	1,042
2016	14	185	220	1,826
2017	18	243	220	1,400
2018 (projected)	21	256	220	1,400

Source: IndexMundi: https://www.indexmundi.com/
Table courtesy of Mutasim Abdelmawla, University of Gezira, Medani, Sudan

of years, sesame and groundnut oil were extracted by means of an animal-drawn mill powered by camel, cow, or horse and based near the areas of crop production. The only further process in traditional Sudanese oil pressing is filtration, which is normally done through a piece of cloth.

In the early 1990s, many mills were improved by the addition of some small-scale mechanical pressing, said Mirghani. "This more advanced mechanical pressing didn't require animal power for pressing, so it spread throughout the country to villages, towns, and cities," he added. At this point, oils still did not undergo any type of further processing other than filtration.

The speed of production increased greatly once larger-scale pressing with mechanical expellers was introduced in the late 1990s, according to Mirghani. This brought fully RBD (refined, bleached, and deodorized) refining to Sudan, primarily of cottonseed oil, groundnut oil, and sunflower oil. Today, only sesame oil still is never fully refined.

"The efficiency of this technology is very low," added Abdalla Ibrahim Mohamed of the United Nations Industrial Development Organization (UNIDO; Khartoum, Sudan). "Approximately 8% to 13% of the oil content is left in the oilseed cakes, compared to solvent extraction technology," he wrote.

Now, Sudan is home to about 250 oil processing mills scattered over many of the country's 25 states, Mohamed reported, with an estimated oilseed capacity of 2.3 million metric tons per year. Of the more than 50 batch refining units, only 20 are in operation, with a total capacity of 100,000 metric tons (MT) of oil. Another five units capable of continuous refining have a total installed capacity of 150,000 MT/year, he concluded.

"A project is underway for a commercial-scale oilseed extraction and oil refining facility, financed by foreign investors," reported Abdalnabi, who noted that it is too early to disclose anything more. That said, it is one more sign that Sudan is close to taking the next steps toward developing its oilseed-related industries.

Research in Sudan

AGRICULTURAL RESEARCH CORPORATION (ARC)

Staffing: 3,552 employees, including 585 researchers and 319 technicians

2018 budget: 424 million Sudanese pounds (about $14.1 million)

ARC, with headquarters in Madani, the capitol of Gezira State in central Sudan, was established in 1967 and is the leading agricultural research and development institute in Sudan. It is the main technical arm of the Ministry of Agriculture and Forestry and is mandated to conduct integrated agricultural research for development. More information is available at www.arcsudan.sd.

NATIONAL OILSEED PROCESSING RESEARCH INSTITUTE (NOPRI)

Staffing: 31 employees, including eight researchers

2018 budget: $123,172

Established in January 1991 as the National Oilseed Processing Research Centre under the aegis of the Faculty of Engineering and Technology of the University of Gezira in Madani, the research institution became NOPRI in 1994. It is the only Sudanese research center solely dedicated to oils and fats research, according to NOPRI's Ismail Abdalnabi. "There are other research centers that work on oils and fats, but they do not have specialized departments for that purpose," he reported.

NOPRI currently has four branches based at universities scattered about Sudan. The main facility is in Madani, which is about 190 kilometers (120 miles) south of Khartoum. The institute has a long history of research on oilseeds; following are some of the projects NOPRI has conducted or is conducting.

Development of glandless varieties of cottonseed—Three glandless varieties (i.e., without gossypol, the toxic phenolic aldehyde found throughout glanded cotton plants) have been identified for commercial-scale production. "Based on the project work plan, the release may take two more seasons," said Abdalnabi.

New crops—NOPRI is currently looking at Argessi (Chrozophora brochiana) and Camelina sativa for edible purposes and kenaf (Hibiscus cannabinus) for industrial uses.

Evaluation of safflower germplasm—A worldwide germplasm collection of 169 accessions of Carthamus spp pro-

vided by the Leibniz-Institute of Plant Genetic and Crop Plant Research in Germany was evaluated by NOPRI in 2014–2015. The selected accessions from two projects are still under trial evaluation, Elfadl Elfadl explained. "On average, the seed yields reported in NOPRI and ARC projects were 562.6 and 561.5 kilograms per hectare, respectively," he noted.

Risk assessment of transgenic Bt cotton—This collaborative project is being conducted by NOPRI and two other research groups at the University of Gezira: the Blue Nile Institute for Communicable Diseases and the Faculty of Agricultural Sciences. It involves a three-generation trial in goats to study the impact of *Bt* cottonseed cake on hormone production, fertility, and the quantity and quality of the animals' milk production, as well as a histopathology study.

Evaluation of tree-borne oilseeds—NOPRI has investigated a number of potential sources, including *Ziziphus mauritiana*, *Adansonia digitata*, and *Grewia tenax*. The *Balanites aegyptiaca* tree, commonly known as the desert date, "is considered the main tree of value as a source of oil in the region," NOPRI's Abdalnabi reported. The research also found that the seed oil content of the three species varied significantly among the six study locations.

Treatment of peanut seed and cake with high aflatoxin levels—This NOPRI program began in February 2016 and is ongoing. An examination of the photocatalytic detoxification of aflatoxins B1 and B2 in naturally contaminated Sudanese peanut oil found that more than 99.4% of aflatoxin B1 and 99.2% of aflatoxin B2 was detoxified within four minutes of treatment at room temperature. Further, there was no alteration noted in the quality and quantity of the oil after treatment, and the treatment process was successfully optimized. (Aflatoxins are poisonous carcinogens that are produced by certain molds that grow in soil; peanut is particularly susceptible.)

Effects of climate change—NOPRI is collaborating with UNIDO Sudan in its project to mitigate the effects of climate change on small-scale sesame and peanut producers and processors.

Assessment of aromatic and medicinal plants—A newly formed NOPRI research group is studying aromatic and medicinal plants such as basil and spearmint, with input from other University of Gezira faculties and institutes. The group also includes traditional healers and herbalists.

MAJOR CHALLENGES LIE AHEAD

Although all the sources consulted for this article expressed optimism that Sudan is entering a new period of growth, they all addressed what they view as the most pressing challenges facing the country's oilseed production and processing industries.

A major challenge to the export of oilseeds is the cost of transportation. Road transportation to Sudan's ports on the Red Sea is costly; exporters have to pay the cost of empty returning vehicles. That cost, added to other transaction expenses such as export fees and taxes, makes the price of exported commodities uncompetitive in world markets, according to Mutasim Abdelmawla, a professor of economics at the University of Gezira.

"Climate change poses significant challenges to Sudan's development priorities including to the vulnerability of the oilseed value chain," added UNIDO's Mohamad. "Historical climate trends indicate a steady rise of temperatures between 0.2°C and 0.4°C per decade from 1960 to 2009. Projected changes in climate indicate that temperatures will increase by 0.5°C to as much as 3°C by 2050, with a more extreme temperature rise in the north. Temperature increases will intensify the impacts of drought through increased evapotranspiration and reduced soil moisture," he concluded.

Further challenges he sees include the need for better access to credit as well as certified seed, seed multiplication, and seed priming; the lack of microdosing of fertilizer; and the need to develop better group storage.

Another growth opportunity lies in the oil filling and packaging sector, Mohamed suggested. Most filling is done manually, he noted, except for "a very few factories. Auxiliary facilities such as laboratories, appropriate warehouses, and storage facilities are inadequate and need modernization. In addition, no waste treatment facilities are available for most of the oil mills."

Adeeb Hayyan, a research fellow in the Nanotechnology & Catalysis Research Centre at the University of Malaysia (Kuala Lumpur) who has frequently lectured in Sudan on biodiesel, sees potential for that industry. The *Inform* editorial advisory committee member notes that NOPRI did proof-of-concept research on *Jatropha curcus* in the 1990s. Ismail Abdalnabi agreed that jatropha oil has the potential to be commercialized in Sudan and Africa. However, "feasibility studies and funding from the Government of Sudan will be needed before the biodiesel industry can progress."

Renewable energy represents yet another oilseed-related industry in Sudan that is at the fulcrum, needing only modest investment and attention to move forward. Given the importance of agriculture to Sudan's economy, diversified development is precisely what the country needs to accelerate growth. In the meantime, NOPRI stands ready—armed with decades of research—to collaborate with other research institutions and advise industry about oilseed crops with the greatest potential. All the elements are in place for development. It remains to be seen whether the potential and opportunity noted by observers actually come to fruition.

Catherine Watkins is a freelance writer based in Champaign, Illinois, USA.

Flying jet plasma: a logistic, powerful catalyzing agent for chemical and biological processes

Wameath S. Abdul-Majeed

- Plasma jets inactivate a wide range of pathogens and are unique in producing reactive chemistry at room temperature.
- Our research group developed a flying jet plasma torch (FJPT) that we used to assist conventional transesterification, to treat raw polymers, and to investigate other applications.
- Relatively low power consumption and environmental impacts give this torch a competitive advantage over well-known glow discharge techniques, suggesting that it could be a friendly new tool for preserving food and catalyzing reactions.

Non-thermal plasma (NTP), also referred to as a cold plasma, is a unique tool with various applications in health care and industry. In NTP, the temperature of electrons typically ranges from 10000 to 250000 Kelvin, while the hot gas exists in the room temperature. These highly energetic electrons produce free radicals from parent molecules in multi-step physical and chemical processes, which gives NTP tremendous destructive capabilities. NTP is currently used in the health sector, and in applications related to environmental remediation, removal of volatile organic pollutants, simultaneous removal of NO*x* and soot in diesel exhaust, and sterilization of air and water.

Plasma jets, a category of NTP which can deliver plasma to distances of up to a few meters, are unique in producing reactive chemistry at room temperature. Such jets have gained considerable attention due to their versatility and low cost of operation. Compared to conventional dielectric barrier discharges (DBD) and corona discharges, jet plasma has a lower shock risk and can penetrate and propagate inside small holes and flexible dielectric tubes, which is quite useful in different applications. For these reasons, our research group developed a flying jet plasma torch (FJPT) as an impetus for a wide spectrum of research (Fig. 1).

Our first endeavor [1] was dedicated to investigating FJPT as a sole and assisting catalyzing agent for biodiesel production from fresh and wasted vegetable oil. Upon examining three reaction schemes, FJPT proved active in assisting conventional tranesterification (yield pro-

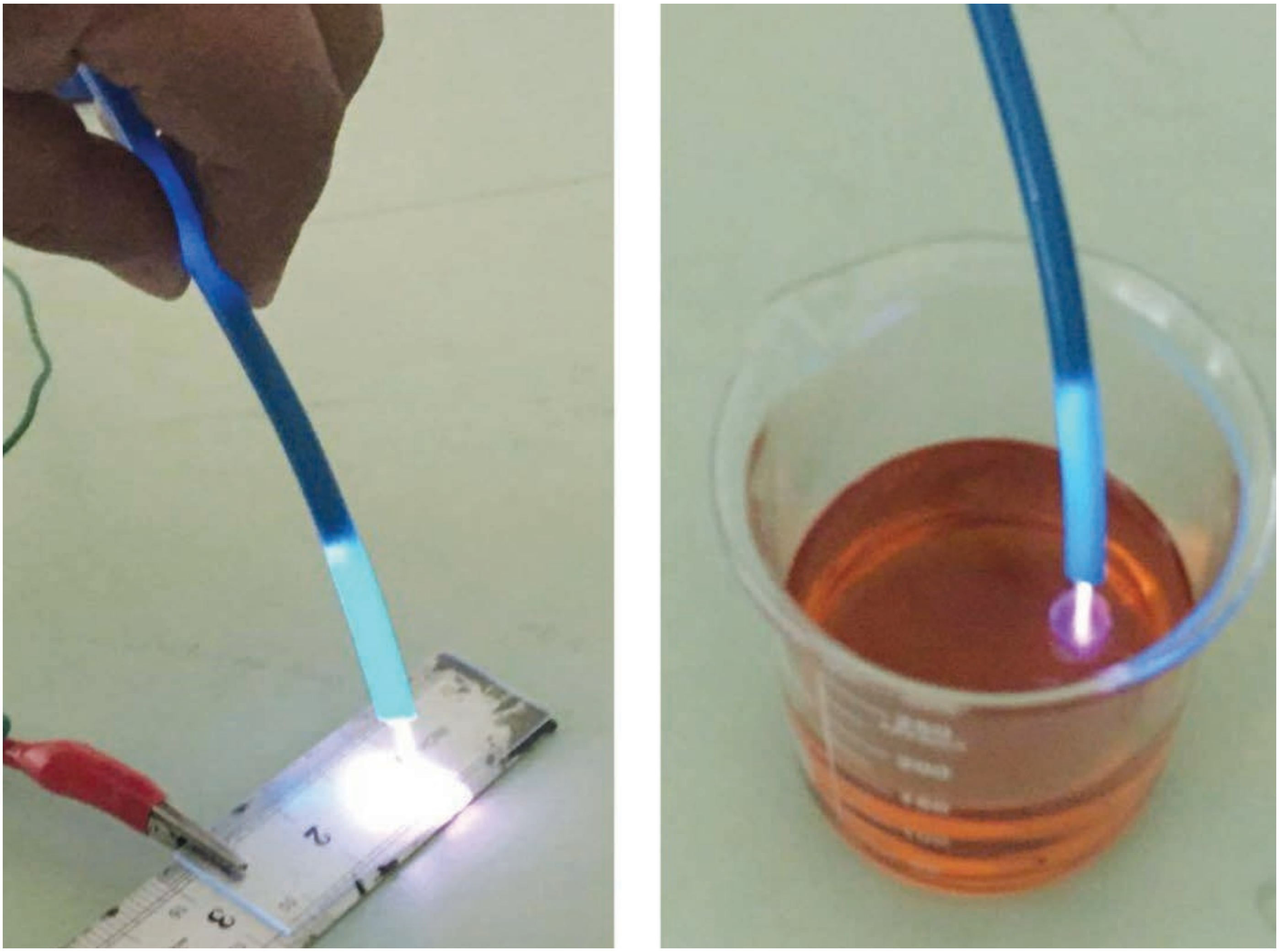

FIG. 1. Pictures of single plasma jet illustrate simple and friendly use

gressed slightly from 93.2 to 95.4%) whereas it proved significant as a sole catalyzing agent, achieving 72.3%. Although a lower yield was achieved, sole plasma catalysis resulted in formation of a considerable amount of saturated methyl esters and a very low amount of co-product; hence, all measured parameters of biodiesel were found promising, in agreement with the commercialization standard [2].

In another path, we examined FJPT for treatment of raw polymers (polypropylene, polystyrene, and polyethylene) before the end use process [3]. Our results indicated that FJPT effectively stimulated topographical modifications and changed the chemical composition of the polymer surface. An increase in roughness of the treated polymers was observed and led to changes in the surface status from hydrophobic to hydrophilic, which is deemed beneficial for external polymeric interactions. We also observed considerable increases in the melt flow index of the treated polymers, which was followed by a very limited decline after 3 days in which no hydrophobic recovery was attained. These observations suggest that FJPT treatment is an active polymer treatment technique that could be used in tandem with other common techniques.

In the health sector, jet plasma has been shown to selectively inactivate a wide range of pathogens, such as bacteria, viruses, fungi, spores, and biofilms. Histological evaluations of skin treated with jet plasma showed no morphological changes, and no significant degree of necrosis or apoptosis were detectable after treatment. Hence, jet plasma has been investigated for skin sterilization, wound healing and tissue regeneration, cancer treatment, malignant cell apoptosis, and blood coagulation [4].

In recent research, we extended the use of FJPT in three paths. In the first, we adopted the flying jet plasma torch to treat spent reforming catalyst. The examined spent catalyst reached exhaustion due to full blockage of surface active sites with deposited amorphous carbon (soot). Upon FJPT treatment, our results have shown considerable elimination of the soot from the active sites; hence, a significant recovery of the catalyst is attained.

In the second path, we utilized FJPT to treat granulated carbon black toward a new path in carbon nanostructure production. Our results showed considerable changes in the treated surface morphology of the granules, as well as changes in chemical structure. Most important, transmission electron microscopy (TEM) images demonstrated formation of carbon nanostructures. In the third route, we investigated the treatment of domestic wastewater samples collected from a waste-

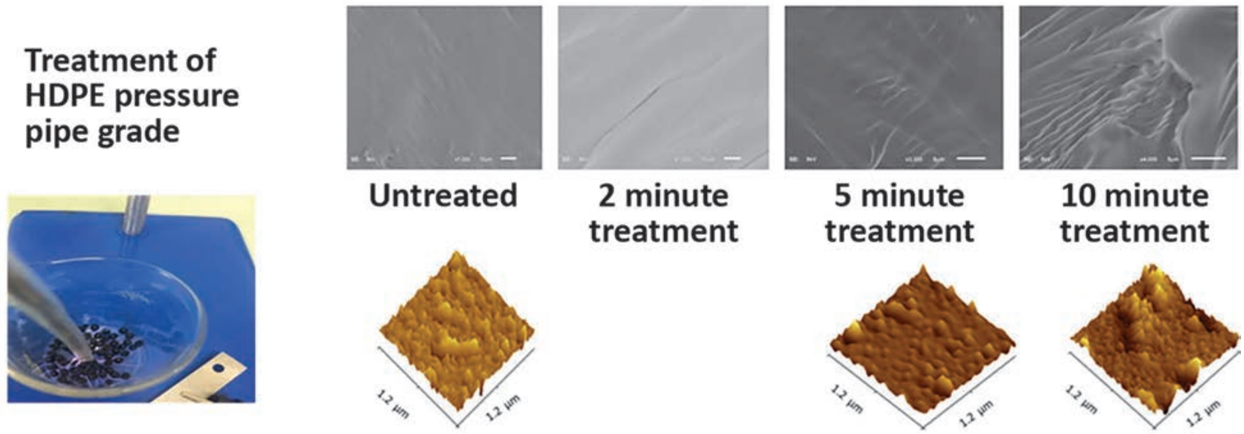

FIG. 2. A flying jet plasma torch (FJPT) was applied to treat raw polymers before the end use process. Specimens of the raw polymers (polypropylene, polystyrene, and two grades of polyethylene) were treated for a few minutes. The examined specimens were analyzed by using scanning electron microscopy, atomic force microscopy, and energy dispersive X-ray spectroscopy. The results indicated that FJPT effectively stimulated the topographical modifications and changed the chemical composition of the surface, which consequently affected the roughness of the surface. Machinability of the examined high-density polyethylene (HDPE) was investigated through measuring the melt mass flow rate index (MFI). The results showed that MFI increased around 30% after 5 min treatments. Longer treatment periods resulted in reductions of MFI attributed to thermal effects. Measured MFI for treated HDPE slightly decreased for 3 days after treatment. Hence, FJPT proved useful for treating polymer surface homogeneously and tailoring the surface chemistry for the required end use. Reprinted with permission from Abdul-Majeed, W.S., *et al.*, Application of novel polymeric surface remediation technique based on flying jet plasma torch, *Ind. Eng. Chem. Res. 56*: 11352–11358, 2017.

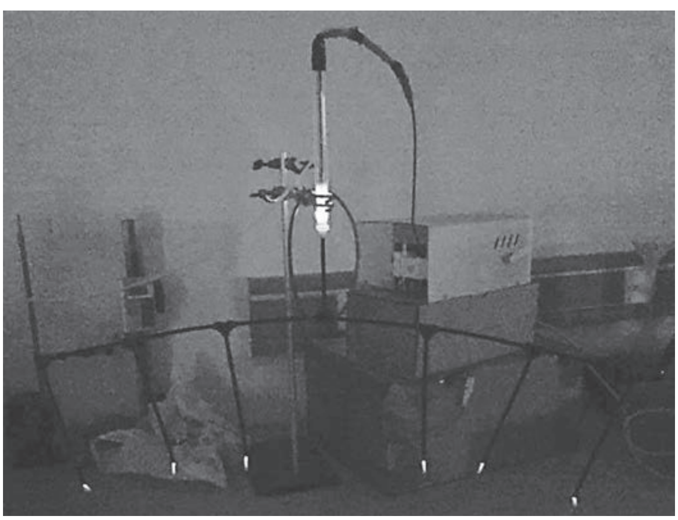

FIG. 3. The multi flying jet plasma torch of six plumes we developed, working simultaneously with the same gas and power source

water treatment plant (WWTP) through FJPT. Our results, as evidence by bacterial growth curves, suggested a significant reduction in biological and chemical oxygen demands, as well as de-activation of microbial activity for several hours.

In all of the investigations described above, the plasma torch was shown to be competitive with other well-known glow discharge techniques in terms of power consumption and environmental impacts. This held true upon extending FJPT to MFJPT (multi flying jet plasma torches), see Fig. 2, when operated by the same power and gas sources. In this respect, examining MFJPT, of six plasma plumes, led to limited power and gas consumption increases of 26% and 14%, respectively. No environmental impacts were observed upon operating FJPT or MFJPT, suggesting that such jet plasma torches could be a friendly tool for preserving food in the food sector, and catalyzing reactions in other sensitive industrial sectors.

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Further reading

[1] Abdul-Majeed, W.S., G.S. Al-Thani, and J.N. Al-Sabahi, Application of flying jet plasma for production of biodiesel fuel from wasted vegetable oil, *Plasma Chem. Plasma Process*. *36*: 1517–1531, 2016.

[2] European biodiesel spec. (EN 14214:2012) has indicated that a biodiesel as a fuel should contain a minimum of 96.5 % ester whereas co-products, such as linoleic acid and methyl ester, should not exceed 12 % in the formed cut.

[3] Abdul-Majeed, W.S., I.M. AL-Handhali, S.H. AL-Yaquobi, and K.O. Al-Riyami, Application of novel polymeric surface remediation technique based on flying jet plasma torch, *Ind. Eng. Chem. Res*. *56*: 11352–11358, 2017.

[4] Abdul-Majeed, W.S., Editorial: Therapeutic non-thermal plasma, significance and challenges. *Letters in Applied NanoBioScience 3* 158, 2014.

A big round of applause

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May 5–8, 2019. AOCS Annual Meeting & Expo, America's Center Convention Complex, St. Louis, Missouri, USA.

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For in-depth details on these and other upcoming meetings, visit http://aocs.org/meetings or contact the AOCS Meetings Department (email: meetings@aocs.org; phone: +1 217-693-4843).

Frog foams and natural protein surfactants

Alan Cooper, Steven J. Vance, Brian O. Smith, and Malcolm W. Kennedy

The mechanical agitation and surfactant activities normally required to create aqueous foams and emulsions are generally damaging to biological systems. Strong shearing forces and interactions at surfaces and other interfaces can disrupt delicate cells and tissues, and can also denature proteins and other biological macromolecules. Conventional ionic/non-ionic detergents and surfactants will disrupt the phospholipid bilayers of cell membranes and can denature proteins. Indeed, various combinations of mechanical disruption and detergent extraction have long been used in laboratory procedures for the homogenization of biological tissues and the extraction and analysis of their various components for biochemical research and other purposes.

Biological foams contain a cocktail of unusual proteins with diverse properties. Such natural foam proteins:

- **have surfactant properties equal to or better than conventional detergents;**
- **reveal new physical principles based on conformational change at interfaces;**
- **illustrate alternative surfactant mechanisms not available to conventional detergents; and**
- **can act synergistically to form and stabilize bio-compatible, hydrated foam structures.**

Consequently, it is perhaps not surprising that natural foams and surfactants are relatively rare in biology. However, there are a few instances where natural biological foams and surfactants have evolved for apparently quite specific purposes, presenting us with an opportunity to explore potentially interesting new physics and physical chemistry of foams in an unusual context. Here we review some of our recent work in Glasgow, Scotland, focusing mainly on the structure and function of some of the proteins that we have identified and characterized as a result of fieldwork and collaborations with colleagues world-wide. Work on other surfactant proteins has been reviewed elsewhere. It is a pleasing historical coincidence that some of the early work on foam physics was initiated in Glasgow by William Thomson (Lord Kelvin). It was here that he devised the classic polyhedral structural model for foams as efficient space-filling entities that has only more recently been superseded by the work of the Dublin group in the form of the Weaire-Phelan model.

Our own interest in biological foams arises out of a natural curiosity for some of the natural foams, in particular frog foam nests, which had not been previously investigated. Foam nesting is one of the strategies that have evolved to allow some species of frogs to provide an appropriate environment for their eggs and embryos in regions of the world where standing water is otherwise rare or transient. These nests are typically found in temporary pools or puddles, on tree branches or on other structures overhanging water, or buried underground (Fig. 1).

As we have shown in earlier work, these water-based foams are made from dilute solutions of proteins and carbohydrates that are very stable under tropical conditions. They resist dehydration, predation, and microbial degradation, and provide a biocompatible environment for the frog eggs, sperm, and developing embryos. As an interesting form of soft matter they merit curiosity-driven investigation in their own right, but they also suggest numerous opportunities for potential

Tungara Frog female *(Engystomops pustulosus)*. Photo by Brian Gratwicke.

FIG. 1. Single nest, *ca.* 10 cm diameter. (Picture credits: Alan Cooper, Rachel Fleming).

practical application. For example, their biocompatibility and moisture retention, coupled with possible antimicrobial resistance suggests possible biomedical applications, including use as temporary wound or burn dressings, surgical cavity filling or matrix for tissue regeneration, or slow-release drug delivery systems. In biotechnology, the surfactant proteins in these foams might be utilized for interfacing to nanoparticle/nanotube/graphene-based components and devices. On a larger scale, as biodegradable aqueous foams they might find application in the treatment of oil spillages, the bioremediation of contaminated areas, or other environmental issues. Plus, as we will indicate later, the surfactant activities of these foam components can help disperse carbon-based nanoparticles, with potential toxicology implications.

But, of course, to implement such potential applications, it is necessary to explore more fully the chemical composition and molecular structures of the foam materials. Here we present some specific examples, describing how details about molecular structures and composition are leading to new insights into biological foam.

RANASPUMINS

The túngara or mud-puddle frog (*Engystomops pustulosus*, previously known as *Physalaemus pustulosus*) is a common ground-dwelling foam-nesting species of Trinidad and surrounding areas of the Caribbean and Central America. Nests are formed during rainy seasons at the edges of temporary puddles or other standing water to provide a stable environment for the developing eggs and embryos.

During amplexus, the female repeatedly produces small batches of eggs along with fluid that the male gathers up, fertilizes and, with an egg-beater like motion of the hind legs, whips up to create the foam nest. Nest building occurs in discrete phases, the first of which is to produce a bubble raft, followed by the construction of a hemispherical foam mass into the center of which the fertilized eggs are deposited.

Depending on conditions, tadpoles develop in the nest over the next few days before escaping into surrounding water, if available. The foam becomes dispersed when the tadpoles hatch, but if the eggs are removed at an early stage then the foam mass remains stable for many days under natural tropical conditions. The nests have typical wet-foam bubble morphologies, comprising 90% air, with the aqueous phase made up of a dilute solution of proteins (1–2 mg ml^{-1}) together with a similar concentration of complex, long-chain carbohydrate molecules.

Although we have found no evidence for fats, lipids, or small detergent-like components in this foam fluid, the natural material has significant aqueous surfactant properties. Natural foam fluid can wet hydrophobic surfaces and reduces surface tension at (total) protein concentrations as low as 10–100 µg ml^{-1}, much more effectively than proteins such as lysozyme or bovine serum albumin (BSA) that are not normally surfactant in their native states (Fig. 2). Biochemical analysis shows that the fluid is made up of a cocktail of proteins, of which six predominate (the "ranaspumins," Rsn-1 to Rsn-6) in the 11–25 kDa size range with intriguing properties. Some, at least, of these proteins can bind hydrophobic fluorescent molecules, and this can be used in 2-photon microscopy techniques to image bubbles in the foam. Amino acid and DNA sequence analysis showed little or no immediate similarity to previously known proteins, but with some potential clues as to their possible function.

RANASPUMIN-2

One protein in particular, ranaspumin-2 (Rsn-2), has a particularly unusual and (so far) unique amino acid sequence, with a highly-charged hydrophilic C-terminus coupled with a significantly non-polar N-terminal sequence that is immediately suggestive of the amphiphilic characteristics of common detergents, but on a much larger molecular scale. Surfactant measurements using pure recombinant Rsn-2 show that this protein is indeed very effective at reducing the surface tension of water at very low concentrations (1–10 microgram ml^{-1}), an order-of-magnitude lower than the natural protein mixture (Fig. 2). And dilute solutions of Rsn-2 can be whipped up to produce foams that appear superficially similar to those seen in natural foam nests, albeit that they tend to collapse more rapidly (in a few hours) than the natural foams (days/weeks).

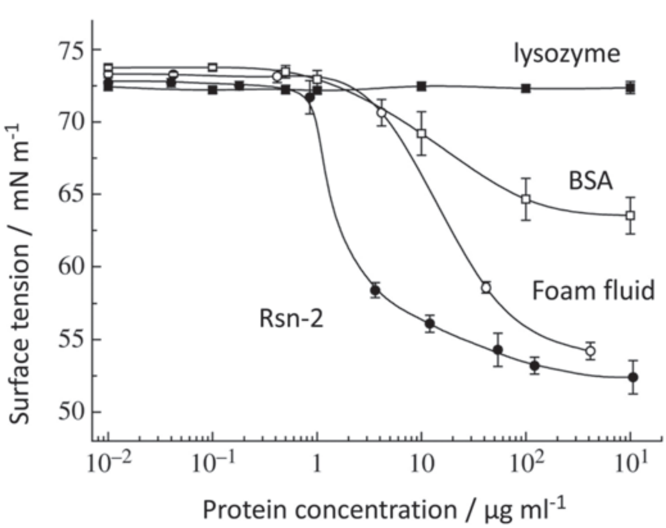

FIG. 2. Aqueous surface tension versus concentration of natural foam fluid compared to pure Rsn-2 and control proteins (BSA, lysozyme).

What is the three-dimensional structure of Rsn-2 that might explain these properties? Using high-resolution nuclear magnetic resonance (NMR) techniques on isotope-enriched samples of recombinant Rsn-2, we have shown that its structure in water is monomeric and typical of a compact, well-folded and highly soluble globular protein.

Surprisingly, despite the amphiphilic nature of the extended polypeptide chain, the NMR structure of the Rsn-2 molecule in bulk aqueous solution shows no obvious hydrophobic patches or other structural features that might be anticipated for a macromolecular surfactant. (In this respect it differs significantly from other surfactant proteins such as hydrophobins that tend to aggregate in solution.) This leads to the suggestion that Rsn-2 might undergo significant conformational change at the air-water or other non-polar interface. Closer inspection of the Rsn-2 structure shows that it is made up of two compact domains, linked by a potential hinge that might allow for a clam-shell-like opening of the structure to present hydrophobic faces of each domain to the interface, whilst maintaining much more polar regions in contact with the aqueous phase. This novel surfactant mechanism neatly solves the conundrum of how to achieve surfactant properties in a macromolecule whilst retaining a compact structure and high solubility in bulk water. It also addresses the issue of biocompatibility, since such large soluble protein molecules are less likely to penetrate and disrupt lipid bilayer membranes in the way that small molecule detergents do.

Although it is not (yet) possible to determine detailed molecular structures of proteins at interfaces, there are several lines of experimental evidence that lend support to the hinge-bending clam-shell model for Rsn-2 surfactant activity. Small-angle neutron reflection (SANR) experiments on the natural foam fluid show a complex air-water interface layer, about 7.5 nm (75 ångstrom) thick, probably made up of several regions of different protein/carbohydrate composition (Fig. 3).

Similar experiments with pure Rsn-2 reveal a much simpler 1 nm (10 ångstrom) monolayer (Fig. 4). This is narrower than would be expected for Rsn-2 in its compact, folded form (diameter *ca*. 2.5 nm), but is compatible with the proposed open clam shell structure at the interface. Infra-red reflection absorption spectroscopy (IRRAS) of characteristic protein amide-I and amide-II bands shows that Rsn-2 retains the anticipated alpha-helix and beta-sheet secondary structure at the air-water interface, with an angular dependence consistent with a planar orientation in the interfacial layer.

In more recent work we have used site-directed mutagenesis to introduce chemical modifications into the Rsn-2 structure in order to investigate the surfactant mechanism. Chemical cross-links can be inserted into the structure by pairwise conversion of selected amino acids to cysteine residues, thereby forming disulphide bonds that might block, or at least

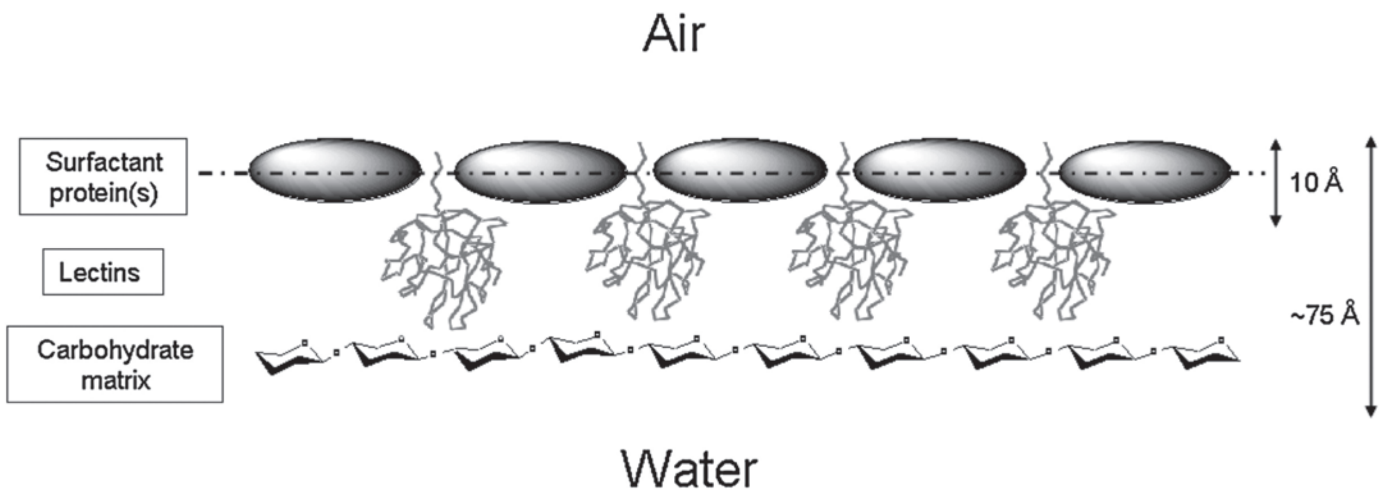

FIG. 3. Small angle neutron reflection profiles of the natural túngara frog foam mixture are consistent with a 3-layer model that, together with additional information, suggests a stable surface structure incorporating surfactant proteins, lectins, and polysaccharides.

partially compromise the hinge-opening mechanism. Results from a series of Rsn-2 disulphide mutants show that in all cases the onset of surfactant activity, as measured by surface tension, is eliminated or significantly reduced in ways that are consistent with inhibition of hinge opening. In other experiments, modification or removal of portions of the hydrophobic N-terminal sequence resulted in diminution or loss of surfactant activity, suggesting that insertion of this end of the polypeptide chain into the non-polar interface provides some of the driving force for the conformational change mechanism. C-terminal modifications show lesser effects, though reductions in the rates of onset of surfactant activity were observed.

Indirect evidence for the hinge-bending mechanism comes from molecular dynamics simulations of what might happen as an Rsn-2 molecule in water approaches a non-polar interface. Consistent with experimental observations, these simulations show that Rsn-2 may adsorb at an air-water interface, with the majority of the non-polar N-terminal residues exposed to the air. The protein secondary structure (alpha-helix, beta-sheet) remains mostly unchanged in these simulations.

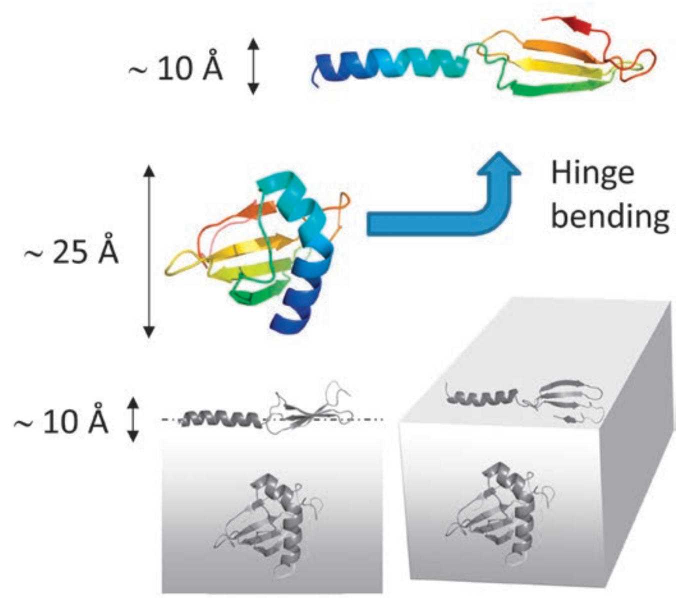

FIG. 4. Neutron reflectivity data for Rsn-2 at the air-water interface in D_2O (□, ◊) or null reflecting water (NRW) (∆) at pH 7 are consistent with a narrow interface layer, compatible with the hinge-bending model.

OTHER RANASPUMINS

Much less is known in detail about the other major proteins in the túngara frog foam nest. Comparison of amino acid sequences suggests that Rsn-1 (like Rsn-2) might be related to cystatins, a class of protease inhibitors that can block the breakdown of protein polypeptides by enzymic degradation. Indeed, in preliminary experiments we have demonstrated potent protease inhibition activity in natural foam material, though this has not yet been pinned down to any specific component in the foam. Recombinant Rsn-1 also reduces aqueous surface tension, though not quite as effectively as Rsn-2. Other ranaspumins in the mix (Rsn-3 to Rsn-6) can be identified as carbohydrate binding proteins (lectins), and this may be related to the possible role of the complex, long-chain carbohydrates in the foam cocktail. Lectin activity and carbohydrate binding has been confirmed in one case (Rsn-4) by demonstration of red blood cell agglutination that is inhibited by lactose and galactose.

From work done so far it would appear that foam nest proteins from other frog species have little in common. Preliminary analysis of proteins from the foam nests of other species, including *Leptodactylus fuscus* (Central and South America), *Limnodynastes peronii* (Australasia) and the Asiatic tree-nesting species *Polypedates leucomystax*and *Rhacophorus arboreus*, shows no resemblance to the túngara ranaspumins.

Likewise, recent structural studies by others of a putative surfactant protein (Lv-RSN-1) from another South American species, *Leptodactylus vastus*, again shows no similarity to the túngara proteins at either the amino acid sequence or protein conformational level, although it does share some sequence similarity with a nest protein from *L. fuscus* and also appears to have a very hydrophobic, unstructured N-terminus that might be related to surfactant function. This general diversity in surfactant protein structure and function has also been noted elsewhere, suggesting that these proteins do not necessarily share a common evolutionary heritage.

FOAM NEST STABILITY

As mentioned above, dilute solutions of pure recombinant Rsn-2 can be whipped up to create foam superficially identical to that seen in natural foam nests. However, these foams in the lab have relatively short-term stability, usually collapsing within an hour or so. This is in marked contrast to what we observe in the wild, where natural frog foam nests survive for many days, and where subsequent collapse or degradation is normally due to mechanical disruption or enzymatic degradation by the tadpoles themselves rather than underlying physics. This suggests that there must be additional factors present in the natural nests that contribute to longer term stability.

These ranaspumin-based aqueous foam liquids are very dilute and low-viscosity, so stability is unlikely to arise from viscous bubble-entrapment/kinetic effects, at least not in this particular species. The stabilization of foams and emulsions by incorporation of small particles has been described in numerous other (mostly non-biological) systems, but again this is unlikely to be a major factor here. Inevitably, given the nature of the biological environment, natural foam nests often contain particulate debris picked up from the surroundings. But this is quite adventitious and varies from site-to-site. Microscopic examination of foam fluid does reveal the presence of small particles (and micro-organisms), but not at a level likely to be relevant to foam stabilization. Moreover, our laboratory-based experiments with captive frogs in pure, de-ionized water show that equally stable foam nests can be produced in the absence of extrinsic factors.

The answer to the stability puzzle must lie in the intrinsic composition of the foam nest cocktail of proteins and carbohydrates. Our current working hypothesis, illustrated in Fig. 3, is that the interfacial layer in the natural foams incorporates other proteins and carbohydrates to form a more stable system. Initial bubble formation is facilitated by the surfactant activity of Rsn-2, but subsequent addition of lectins (Rsn-3/4/5/6?) builds up a more complex layer that may then also be reinforced by binding to long-chain branched polysaccharide molecules. At least two of the ranaspumin lectins (Rsn-3 and Rsn-5) have amino acid sequences possessing moderately hydrophobic N-terminal tails that might serve to anchor and orient these molecules in the interface. Further binding to an underlying carbohydrate layer would create a mechanically stable, water-retaining foam matrix with a thickness compatible with the SANR data.

It should be borne in mind that foam nest stability in the wild will depend on factors other than plain physics—in particular, resistance to predation and microbial degradation is important in the biological context. Lectins similar to Rsn-3/4/5/6 can inhibit microbial growth by agglutination of cells and can also act as anti-feedants in higher organisms. Lectins and protease inhibitors in plants and detergent secretions can act as defenses against insect attack. It seems likely, therefore, that a combination of these properties found in the nest proteins are sufficient, acting synergistically, to stabilize the nest against environmental and biological challenges for just long enough for the purpose of tadpole development without replenishment.

RANASMURFIN

Many tree frogs also build foam nests, usually overhanging water on branches or other adjacent structures. One example of this is *P. leucomystax*, a common tree frog from Malaysia and surrounding regions, whose nests have the intriguing property of developing a blue/green pigmentation over time. This coloration is due to an unusual zinc-containing protein, designated ranasmurfin (Rsf), which crystallizes readily after purification from natural nest material. X-ray diffraction from these intense blue crystals was used to determine the protein structure to high resolution, revealing a 26 kDa dimeric structure incorporating various unusual chemical modifications.

The function of this protein is not yet known, but it is present in the foam at relatively high levels suggesting that it may be involved, at least in part, in foam stabilization, adhesion, or other mechanical properties, possibly utilizing its chemical cross-linking properties. Other possibilities include camouflage and/or UV protection. The total protein concentration in *P. leucomystax* foam fluid is 2–4 mg ml^{-1}, depending on sample, with about 1–1.5 mg ml^{-1} carbohydrate (Rosalind Tan, Malcolm Kennedy & Alan Cooper, unpublished). The foam itself is rather sticky and viscous in this species, and is probably kinetically stabilized by viscous incorporation of bubbles rather than by specific surfactants.

The *Engystomops pustulosus* ("túngara" or "mud puddle frog")

LATHERIN

Latherin is a protein found in the sweat of horses and is one of the first proteins shown to have strong surfactant activity in its native state and adsorbs well to waxy surfaces. Its function is thought to be to act as a wetting agent to facilitate evaporative cooling from the oily surface of the pelt, where it is probably the contributing factor to the foaming/frothing of horse sweat during vigorous exercise. It is also found in horse saliva and is a member of the palate, lung, nasal epithelium clone (PLUNC) family of innate immunity proteins that are abundant in the oral cavity and saliva of mammals and which have surfactant, bacteriostatic and antibiofilm properties. Latherin is characterized by an unusually high content of non-polar amino acids, especially leucine which makes up about 24% of the latherin total, compared to an average of around 10% for most other proteins.

Although such abundance of non-polar amino acids might be thought to be the source of latherin surfactant activity, this is not immediately apparent in its three-dimensional structure in solution. The protein is a monomer in solution, with a structure, as determined by NMR, comprising a slightly curved cylindrical structure in a "super-roll" motif made up of a four-stranded anti-parallel beta-sheet and two opposing alpha-helices which twist along the long axis of the cylinder. Although there is no obvious hydrophobic patch on the protein surface, one end of the molecule does have prominent, flexible polypeptide loops that contain a number of apolar amino acid side chains. This suggests a plausible mechanism for surfactant activity in which the molecule is first localized to the non-polar interface via these loops, and then unfolds and flattens to expose its hydrophobic interior to the air or non-polar surface. As was also the case with Rsn-2, this is supported by SANR experiments that indicate that latherin forms a thin, ca. 1 nm (10 ångstrom) air-water interface layer consistent with unwrapping or unfolding of the protein.

APPLICATIONS AND IMPLICATIONS

Despite the promising range of potential applications, relatively few examples exploiting natural protein surfactants have been reported so far. Applications in commercial biomedical, food technology, and related areas may be hindered by a reluctance to embark on the necessary investigation of potential toxicology and allergenicity implications for these biomaterials from unusual sources. Nonetheless, other potential applications are making progress. Recombinant Rsn-2 has been used by others to form a stable foam matrix incorporating components for a cell-free artificial photosynthesis system. In unrelated work, we have taken advantage of recombinant protein technology to construct a fluorescent Rsn-2/iLov protein conjugate, demonstrating how the surfactant properties can be attached to other functional proteins.

This ability to engineer recombinant proteins is particularly relevant for possible applications in nanotechnology involving interface of biological macromolecules to other components. For example, as we have recently shown both latherin and Rsn-2 can be used to solubilize or disperse C_{60}-fullerene and bind to carbon nanotubes in water. The next step (work in progress) is to utilize this binding to attach other functional proteins to the carbon (nanotube, graphene) surface.

The aqueous dispersion/solubilization of hydrophobic particles by natural surfactant proteins demonstrated here has potential toxicology implications, indicating a possible route for biological uptake of carbon nanotubes and other nanoparticles. This might, for instance, be of potential concern for nanoparticle uptake by proteins related to latherin (PLUNCs) that are present in the human respiratory tract and elsewhere.

Alan Cooper is an honorary senior research fellow in the School of Chemistry at the University of Glasgow, Scotland. He can be contacted at Alan.Cooper@glasgow.ac.uk. Steven J. Vance, Brian O. Smith, and Malcom W. Kennedy are also at the University of Glasgow.

IN MEMORIAM

Giants of the past: Bernard F. Szuhaj (1942–2018)

G.R. List

Bernard (Bernie) Szuhaj was born in Washington Township, Pennsylvania. He graduated from Washington High School in 1960, and continued his education at Penn State University, where he earned B.S., M.S., and Ph.D. degrees in biochemistry in 1964, 1966, and 1969, respectively. While attending Penn State, Bernie married his childhood sweetheart, Carole, in 1964. Three sons were born into the Szuhaj family: twin boys Matt and Tim, and Bernard Jr. After completing his doctorate, Bernie moved his young family to Chicago, Illinois, embarking on a 35-year career at Central Soya, where he became director of research with a staff of 80 scientists and support personnel. Bernie retired in 2003 after a long and productive career, so that he could care for Carole.

Bernie's research focus centered on the food, industrial, and pharmaceutical uses of lecithin—a major by-product of soybean processing. He held a number of US patents in these areas. I first met Bernie in 1973, when he chaired a session at the AOCS meeting in Chicago. In 1980, I was invited to organize and chair a session on lecithin at the Joint IFS /AOCS meeting in New York City. I turned to Bernie for help in finding speakers and co-chairing the session. The symposium featured eight well-known speakers, and the papers were published in a special issue of the *Journal of the American Chemists' Society (JAOCS)* in January 1981. Bernie suggested that enough new information warranted a book on lecithin, since the last book on the topic was published in 1951 (*The Phosphatides*, Wittcoff).

We began by inviting authors, and by 1985, Lecithins, edited by Bernie and your author, was published by AOCS Press and became a best seller. The book is referenced in many patent applications. In 1988, Bernie organized a short course on lecithin held in Phoenix, Arizona. Again, Bernie envisioned a book resulting from the short course. By 1989, *Lecithins: Sources, Manufacture and Uses* was published by AOCS Press. Bernie was not finished publishing books on lecithin. In 1996, a world conference on phospholipids was held in Brussels, Belgium. Several lectures covered the health-promoting properties of choline and phospholipids. Stephen Ziesel, along with Bernie, edited the proceedings, which were published under the title, *Choline, Phospholipids, Health and Disease* (AOCS Press, 1998).

Bernie was a founding member of the AOCS Phospholipid Division and served in all its leadership positions. The International Lecithin and Phospholipid Society (ILPS) was incorporated in the United States in 1991. Bernie played a key role in the establishment of the society and served as its first president. In 2013, the society was transferred to Europe, where most of the meetings are held.

Bernie was active in civic affairs as a Cub Scout leader, a baseball coach, and a science fair advisor and judge. Throughout his life, Bernie was a devout church member, first at the Sacred Heart Catholic Church in Lombard, Illinois, and later at St. Vincent Catholic Church in Fort Wayne, Indiana.

Bernie was a consummate professional in every way, as well as a world authority on lecithin. Few, if any, had more impact on the lecithin industry, and he was highly respected for his modest, unassuming demeanor and friendly, helpful disposition. For his exemplary service to AOCS and his many technical accomplishments, Bernie was elected as an AOCS Fellow in 2013. He passed away on September 14, 2018.

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

CRISPR crops: Should gene-edited crops be regulated like GMO?

Rebecca Guenard

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

As temperatures warm and populations grow, the agricultural industry faces ever-increasing challenges to produce an adequate food supply. The potential to overcome these challenges lies within gene-editing, a new technology that allows scientists to rewrite pieces of a plant's genetic code, making plants hardier, more productive, and consumer friendly. However, opposing regulatory rulings make it less likely that the benefits of this promising new technology will be realized worldwide.

US regulators, along with those in other countries like China and Canada, have chosen to view gene-editing as an extension of traditional plant breeding. In March 2018, the US Department of Agriculture (USDA) announced that it will not regulate plants that have been modified through genome editing, since plant varieties produced by gene-editing are "indistinguishable from those developed through traditional breeding methods." The announcement explained that genome editing is a much faster form of traditional breeding that can introduce new plant traits more quickly and precisely, potentially saving years or even decades in bringing needed new varieties to farmers (https://tinyurl.com/USDA-CRISPR-statement).

In contrast, four months after the USDA announcement the Court of Justice of the European Union (EUJ) ruled that gene-edited crops would be designated genetically modified organisms (GMO) and subject to similar strict regulatory restrictions (C-528/16, https://tinyurl.com/eu-CRISPR-ruling). In a statement the EUJ said, "The Court of Justice takes the view, first of all, that organisms obtained by mutagenesis [CRISPR, *et al.*] are GMOs within the meaning of the GMO Directive, in so far as the techniques and methods of mutagenesis alter the genetic material of an organism in a way that does not occur naturally."

The EU ruling was a surprise. Many researchers had assumed that the EUJ, like other regulatory bodies, would not require regulations for gene-editing since, unlike GMO, foreign DNA cannot be detected in the resulting plants. Gene-editing techniques, such as CRISPR, work like an industrial sorting machine eliminating bad apples from a bunch. Any genes that code for unwanted traits like disease can be kicked out of a genome with an editing tool like the Cas9 protein. This protein is an enzyme that clips the unwanted section of DNA and either repairs or replaces it (https://tinyurl.com/CRISPR-explained). Once CRISPRed, a fraction of a plant's progeny will contain only the corrected code and will continue to grow as if the error never existed. Its DNA will replicate following this refined set of genetic instructions. So far, researchers have found no trace of the original genetic code or of the Cas 9 editing tool in later generations of a plant.

The EUJ's ruling immediately affects Professor Johnathan Napier of Rothamsted Research center, who is in the middle of a field trial for the United Kingdom's first gene-edited crop. Napier has spent 20 years tinkering with plant biology to produce long-chain omega-3 oils, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). These oils are commonly found in cold-water fish that consume marine microalgae, but Napier seeks to create a terrestrial source. Napier's project involves genetic modification. He takes genes from an omega-3 generating algae and incorporates them into the DNA of a native European plant, camelina but he is also studying the gene-editing technique known as CRISPR. When Napier submitted the EU regulatory filing for this GMO research, he included a collaborator's side project that involved using CRISPR to increase production of oleic acid in camelina.

"It would have been great if using CRISPR wouldn't have been considered genetically modified. You could have just made some tweaks or improvements without having to go through another cycle of regulatory approval," says Napier. "But now it is clear, at least in Europe, that is not going to be the case."

The future of Napier's field experiments is uncertain while he awaits clarification on how to implement the EUJ ruling. But he won't give up on conducting field trials despite the six-month process necessary to receive approval on an experiment. He says field trials are critical to understanding the effects of gene-editing, and he is already accustomed to the system of filing and waiting.

Still, he acknowledges what an obstacle the regulation is for innovation. "If there is a huge regulatory burden that prevents that technology from being used then nobody is going to fund that research," he says. "They will say, 'Well, it will never get used so why should we pay for this?'" He points out that only well-funded research institutions in Europe like his can afford to conduct fundamental research on gene-editing. "It's not even remotely close to being commercialized," he says. "There would be much more burden to do an application for commercialization."

Major corporate players have already expressed that they will not pursue CRISPR technology because of the hassle of the EU regulation. According to *Reuters*, Bayer's Chief Executive Officer Werner Baumann made a statement that the company would not develop genetically modified crops for use in Europe (https://tinyurl.com/companies-decide-against-GE). The report also indicates that BASF moved its plant research operations to the United States from Germany out of frustration with EU regulatory hurdles. Having recently acquired Bayer's seed business earlier this year BASF, again must deal with the EU. Three days after the ruling, BASF Chief Executive Martin Brudermueller told business analysts, "As we run a global platform, it would mean that basically these applications of these instruments would not be used in Europe and Germany. So overall, that does not impact us as a company too much, but as a European, I'm worried about what that means to the Europeans."

Dan Voytas, a genetics and cell biology professor at the University of Minnesota, cofounded the food ingredient company Calyxt in St. Paul, Minnesota, USA, in 2010. Less than a decade later the company will be the first to bring a gene-edited product to market, a high-oleic soybean oil. "The US is very open to the technology, and the regulators are actually coming up with policy that is both science-based and product focused," says Voytas. He says that if he edited a gene to introduce a variation that already exists in nature, there would be no way to distinguish the edited plant from the natural one. Yet, the EU would require the same arduous filing process required for GMO.

"If you look at the first wave of biotech—the transgenic plants, traditional GMOs—they are only corn, soybean, cotton, canola, just a handful of the big row crops," says Voytas. "The regulation was so expensive that no one used it on strawberries or tomatoes." With more science-minded regulations on gene-editing in the United States, he believes that the products of gene-editing will not be so limited. "I think you are just going to see in the coming years a tsunami of products that never happened in the traditional GMO realm."

Voytas emphasizes that extensive testing is performed to determine that the genetic integrity of their plants has not been compromised before any of their products are considered for market testing. "Sometimes genes act in multiple ways in the life of an organism," he says. "Sometimes there are unexpected consequences of editing a gene." It is that possibility that has some critics pushing back against the development of gene-edited products.

Researchers have raised concerns about experimental results that indicate CRISPR is not the precise editing tool some believe it to be, especially as it pertains to human applications. Scientists have discovered that some proteins inside cells block CRISPR's edits (doi:10.7554/eLife.12677, **doi:10.1038/nmicrobiol.2016.85,** https://tinyurl.com/rolled-up-genes). Researchers are also beginning to understand an issue they observed early in CRISPR's inception; the Cas9 protein damages the genome when it makes its cuts (https://doi.org/10.1038/nbt.4192). And there are still fears that the technology will lead to cancer (doi:10.1038/s41591-018-0050-6, doi:10.1038/s41591-018-0049-z) when used to treat human diseases.

These concerns could cause some to believe that using the editing tool on crops should require regulation. For Napier, it's the opposite. "I think that it is a game changing technology," he says. "We are still really at the start of what we can imaging using it for." Only by reducing regulation can scientists uncover the limits of CRISPR's capabilities.

As he has done in the past, Napier will sometimes collaborate with US and Canadian companies to expedite his research process. However, he would prefer to continue his work in the United Kingdom, since environmental conditions differ from country to country, region to region. It's possible that as the UK prepares to leave the EU early next year the country will rollback regulations (https://tinyurl.com/GE-after-Brexit). In the meantime, his gene-editing field trials are coming to an end with some interesting results. "It turns out that if we have a plant that is making high levels of oleic acid, they don't perform very well in the field," he says.

As is often the case, experiments lead to more questions. Looking for an answer to even one of those questions at his field laboratory in the UK will require that Napier first go through the tedious process of regulatory approval. To him, any forward progress is a benefit, no matter how slow.

"We are going to need to feed 9, 10 billion people in less than 30 years, so we really need every possible trick that we have," says Napier. "It puts people trying to drive innovation forward in Europe at a big disadvantage with people in North America, South America, China, and everywhere else who are using this technology."

Voytas mirrors this sentiment. "I understand that Africa often follows Europe's lead in terms of regulations," he says. "In developing countries, where food securities are an issue, this technology could have a positive impact. Climates are changing, crops are put under new stresses, populations are burgeoning, and now they may not be able to use this very powerful tool."

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

United States, Canada issue chemical assessment collaboration framework

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

Kelly Franklin

Canada and the United States have released a framework to improve their collaboration on the risk assessment of chemicals.

A joint product from the US Environmental Protection Agency (EPA), Environment and Climate Change Canada (ECCC), and Health Canada (HC), the "assessment collaboration framework" will enable "enhanced alignment" on the risk-based assessment of chemicals, including on identifying priorities, information gathering, comparing assessment methodologies, and work sharing.

According to the document, the EPA, ECCC, and HC have common policy objectives under the Toxic Substances Control Act (TSCA) and the Canadian Environmental Protection Act (Cepa) to reduce the risk of chemicals to human health and the environment. They also share "similar principles" regarding the assessment of chemicals.

The framework, therefore, aims to "facilitate and enhance collaboration" between the three agencies on risk assessment efforts, working within their respective statutory requirements.

The document reflects plans for collaboration on science and practices, and also to engage stakeholders and the public. This may include the use of a stakeholder advisory group, as well as the development of ad hoc or specialized groups focused on specific issues.

Information sharing between the governments, while protecting confidential business information (CBI), is also a named priority.

ROLLING WORK PLAN

The framework is intended to be flexible and remain valid for an "indeterminate period." But alongside it, the agencies appended a rolling "work plan" of current collaborative activities that cover:

- risk assessment methodologies for new and existing chemicals, including alternatives to animal testing and sharing guidance and tools;
- data evaluation activities, with plans for Canada to give the United States more information on robust study summaries;
- priority setting, with a focus on lessons learned from previously used prioritization approaches; and
- information sharing, including comparing data gathering approaches and exchanging environmental monitoring data for certain substances.

The workplan also highlights plans to share work on the solvents N-methylpyrrolidone (NMP) and 1-bromopropane, which are both among the first ten substances being evaluated under the amended TSCA.

It also indicates "ongoing" inter-agency peer review of Canada's nanotechnology framework, and of the United State's strategic plan on alternatives to animal testing.

A timeline for inter-agency review of individual risk assessments remains undetermined.

The work comes under "stream B" of the US-Canada Regulatory Cooperation Council (RCC) chemicals management work plan, which centers on risk assessment.

"Stream A" focuses on significant new activity (Snac) provisions and significant new use rules (Snurs)—Canada and the United State's respective instruments for controlling risks that may result from new chemicals or new applications of existing chemicals.

In 2018, the two governments committed to "continue their dialogue on regulatory issues and pursue shared regulatory outcomes that reduce trade impediments, reduce costs, increase economic efficiency, and streamline regulations without compromising health, safety, and environmental standards," according to the framework document.

Kelly Franklin is North America editor for Chemical Watch.

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LATAM: Plant protein in bakery, snacks, and sports nutrition

Leslie Kleiner

Plant protein fortification in bakery, snacks, and sports nutrition is a growing trend in the United States, and is also starting to grow in LATAM. To better understand the role of plant proteins in these categories, I consulted Innova Market Insights (www.innovadatabase.com).

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

The search focused on products that were launched, reformulated, repackaged, and/or present on supermarket shelves for the past three years, and also included plant protein in the ingredient list. Please note that the use of plant-based protein does not exclude combinations of plant and dairy protein in the same application. Here is what I found, in Q&A format.

Q: Where in LATAM do we see the largest penetration of plant proteins in bakery, snacks, and sports nutrition for the 2015–2018 period?

Figure 1 shows the distribution of products with plant protein; Brazil and Mexico are the largest players. The plant proteins listed for these products combined are (in alphabetical order): almond protein, amaranth protein, broad bean concentrate, broad bean hydrolysate, broad bean isolate, broad bean protein, brown rice protein, buckwheat protein, chia protein, chia seed protein, chickpea protein, corn protein, flaxseed protein, green bean protein, hydrolyzed corn protein, hydrolyzed potato protein, hydrolyzed rice protein, hydrolyzed wheat protein, lupin protein, mung bean protein, oat protein, pea protein, pea protein concentrate, pea protein hydrolysate, pea protein isolate, peanut protein, potato protein, pumpkin seed protein, quinoa protein, rehydrated pea protein, rice bran protein, rice protein, rice protein concentrate, rice protein isolate, sesame protein, soy protein, soy protein concentrate, soy protein hydrolysate, soy protein isolate, soy protein nuggets, soy protein textured, spelt protein, spirulina protein, textured pea protein, textured wheat protein, tofu protein, water lentil protein, wheat bran protein, wheat protein, white bean protein, yellow pea protein

For the products encountered in Brazil (264), the market sub-segmentation (one or more categories in the product) was as follows: added protein (67), allergen (125), ethical packaging (42), gluten-free (120), GMO-free (6), lactose free (47), no trans fats (25), sports and recovery (147), vegan (17).

For the products encountered in Mexico (192), the market sub-segmentation was as follows: added protein (13), allergen (39), ethical packaging (107), gluten-free (34), GMO-free (6), lactose free (14), no trans fats (51), sports and recovery (11), vegan (13).

Q: Soy protein has an established presence as a source of plant protein. Are there other plant proteins that are also becoming well established?

Plant proteins such as pea and rice are starting to emerge, and have the advantage of not being listed as major allergens. However, soy protein continues to be the most widely used plant protein in the region for the categories studied. In fact, for all bakery, snacks, and sports nutrition products in the region, during the 2015–2018 period, the plant proteins listed in the ingredients were as follows (these were used either alone or combined): soy protein (263), soy protein isolate (197), soy protein hydrolysate (81), wheat protein (45), hydrolyzed wheat protein (38), hydrolyzed corn protein (37), pea protein (22), soy protein concentrate (21), soy protein textured (21), soy protein nuggets (20), rice protein (15), pea protein isolate (11), corn protein (10), amaranth protein (7), brown rice protein (6), lupin protein (4), potato protein (4), pea protein concentrate (3), quinoa protein (3), almond protein (2), oat protein (2), pumpkin seed protein (2), rice protein concentrate (1), textured wheat protein (1).

Q: Which are examples of products with pea protein and which of products with rice protein?

Examples of products with pea protein are: Clif Bar Nut Butter Filled Energy Bar With Coconut And Almond Butter (Guatemala, May 2018, contains nuts and organic pea protein to provide 14 g of protein/ 50 g serving size); Flap Jacked Protein Pancake And Baking Mix Buttermilk Flavor (Guatemala, April 2018, contains whey protein isolate and organic pea protein to provide 20 g of protein/ serving size). Mestemacher Integral Bread Loaf (Brazil, April 2017, combines wheat and pea protein to deliver protein enriched bread).

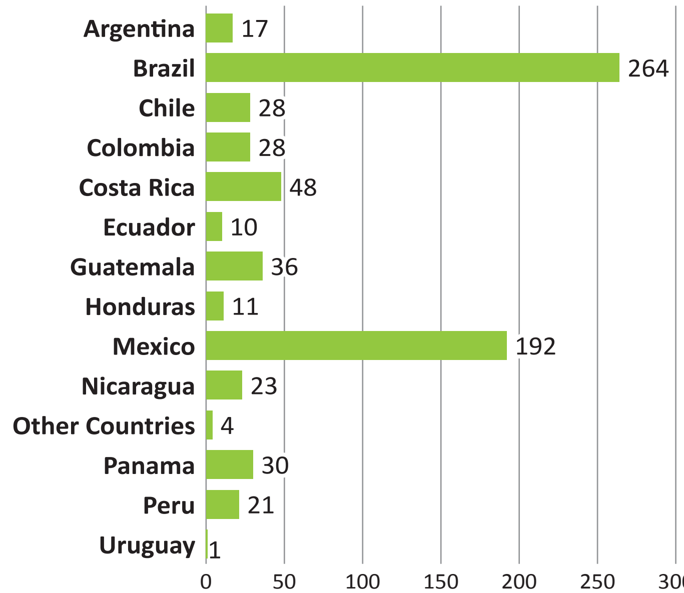

FIG. 1. Product launches for bakery, snacks, and sports nutrition including plant proteins (2015–2018)

Examples of products with rice protein are: Max Titanium Snack Protein Cebola E Salsa: Protein Snack With Onion And Parsley Flavor (Brazil, January 2018, contains rice and corn protein, and delivers 10 g protein per pack); One Nature Organic Protein Powder With Natural Flavor (Mexico, May 2018, organic protein powder mix with rice, pea, and other plant proteins, to provide 32 g protein / 40 g serving size—sports nutrition application).

Latin America Update is produced by Leslie Kleiner, R&D Project Coordinator in Confectionery Applications at Roquette America, Geneva, Illinois, USA, and a contributing editor of *Inform*. She can be reached at LESLIE.KLEINER@roquette.com.

PATENTS

Bio-based synthetic fluid

Abhari, R., *et al*., REG Synthetic Fuels, LLC, US10011783, July 3, 2018

A method is provided involving altering the viscosity of bio-derived paraffins to produce a paraffinic fluid, where the altering step includes oligomerizing the bio-derived paraffins to produce an oligomerized product; the bio-derived paraffins include a hydrodeoxygenated product produced by hydrodeoxygenating a bio-based feed where the bio-based feed includes bio-derived fatty acids, fatty acid esters, or a combination thereof; the bio-derived paraffins include n-paraffins; and the n-paraffins have a biodegradability of at least 40% after about 23 days of exposure to microorganisms. Also provided are methods of protecting and/or cleaning a substance by applying the paraffinic fluid.

Pre-spray emulsions and powders containing non-polar compounds

Bromley, P.J., Virun, Inc., US10016363, July 10, 2018

Provided are compositions and methods for producing water-soluble powders that contain additives such as essential fatty acids, including omega-3 fatty acids, omega-6 fatty acids, conjugated fatty acids, and other fatty acids; phytochemicals, including phytosterols; other oils; and coenzymes, including coenzyme Q10, and other oil-based additives.

Modified soybean oil-extended SBR compounds and vulcanizates filled with carbon black

Soucek, M., *et al*., The University of Akron, US10035903, July 31, 2018

Rubber formulations include a rubber and a norbornylized seed oil. The norbornylized seed oil is a seed oil that is modified with norbornylene groups. The norbornylized seed oil can replace petroleum oils currently often used in rubber formulations. A process for creating a rubber formulation includes the steps of adding a norbornylized seed oil to a rubber, wherein the norbornylized seed oil is a seed oil modified with norbornylene groups.

Pressure-sensitive adhesives based on fatty acids

Li, K., *et al*., Oregon State University, US10030182, July 24, 2018

A method for making a pressure sensitive adhesive comprising: (a) making at least one epoxidized fatty acid from at least one plant oil, marine oil, other ester of unsaturated fatty acid, or a mixture thereof; and (b) polymerizing the at least one epoxidized fatty acid to produce a pressure sensitive adhesive. A method for making a pressure sensitive adhesive comprising: (a) making epoxidized oleic acid, an epoxidized linoleic acid (including fully and partially epoxidized linoleic acid), or an epoxidized linolenic acid (including fully and partially epoxidized linolenic acid) from at least one plant oil, marine oil, other ester of unsaturated fatty acid, or making mixture thereof; and (b) polymerizing the epoxidized oleic acid, the epoxidized linoleic acid, the epoxidized linolenic acid, or the mixture thereof to produce a pressure sensitive adhesive.

Method of cleaning a diesel particulate filter

Dixon, D.R., *et al*., Savannah River Nuclear Solutions, LLC, US10029246, July 24, 2018

The present invention is directed to a method of cleaning a diesel particulate filter. The method requires a step of contacting the diesel particulate filter with a cleaning composition containing one or more fatty acids or a derivative thereof. The one or more fatty acids or a derivative thereof may include a natural oil, such as a plant-based oil.

Compositions comprising a fatty acid oil mixture and a free fatty acid, and methods and uses thereof

Hustvedt, S.O., *et al*., Pronova Biopharma Norge AS, US10028928, July 24, 2018

Compositions comprising a fatty acid oil mixture and at least one free fatty acid, and uses thereof are disclosed. Further disclosed are preconcentrates capable of forming a self-nanoemulsifying drug delivery system (SNEDDS), a self-microemulsifying drug delivery system (SMEDDS), or self-emulsifying drug delivery systems (SEDDS) in an aqueous solution. Preferred fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a form chosen from ethyl ester and triglyceride.

Separation of oil-water mixtures using nanotechnology

Lead, J., University of South Carolina, US10071919, September 11, 2018

Methods for extracting oil from a multiphasic liquid are provided. The method can comprise: introducing the multiphasic liquid to a plurality of nanoparticles, and allowing oil in the multiphasic liquid to be adsorbed by the polymeric shell. The nanoparticles comprise a core and a polymeric shell. The method can further comprise removing the nanoparticles from the multiphasic liquid, and/or recovering the oil adsorbed by the polymeric shell after removing the nanoparticles from the multiphasic liquid. The multiphasic liquid can comprise oil and water (e.g., oil and sea water, such as sea water in an area of an oil spill), stomach fluid and a food-grade oil (e.g., olive oil, vegetable oil, canola oil, or a mixture thereof), or other multiphasic liquids having an oil component.

Sprayable topical carrier and composition comprising phosphatidylcholine

Herslof, B., *et al.*, Lipidor AB, US10034943, July 31, 2018

A pharmaceutical or cosmetic carrier for topical administration substantially consists of phosphatidylcholine, monoglyceride, fatty acid ester of C.sub.1-C.sub.3 alcohol; volatile solvent selected from ethanol and its combinations with C.sub.3-C.sub.4 alcohol and/or volatile silicone oil. Also disclosed are pharmaceutical and cosmetic compositions comprising the carrier and pharmaceutically or cosmetically active agent(s).

Certain triacylglycerols as crystallization depressants

Narine, S., *et al.*, Trent University, US10047313, August 14, 2018

This application relates to the polymorphism and microstructure of certain triacylglycerols and fatty acid methyl esters, including 1,2-dioleoyl-3-stearoyl glycerol and methyl stearate, and how the properties of these individual components in a biodiesel fuel, as well as their combined mixtures, helps understand the fundamental mechanisms of their crystallization so as to design biodiesel fuels with improved low temperature characteristics.

Microalgal compositions and uses thereof

Schiff-Deb, C., *et al.*, Corbion Biotech, Inc., US10053646, August 21, 2018

Provided are microalgal compositions and methods for their use. The microalgal compositions include lubricants that find use in industrial and other applications.

Heavy crude oil viscosity reducer

Bello, C., Oil & Gas Tech Enterprises C.V., US10053641, August 21, 2018

A viscosity reducer based on vegetable extracts of natural origin is disclosed. The vegetable extracts include a mixture of phosphoglycerides and vegetable oils. A method of reducing the viscosity in heavy and extra heavy crude oil using the viscosity reducer is also disclosed. No aromatic base solvents are needed. A reduction in diluent usage is achieved using the viscosity reducer based on vegetable extracts. The viscosity reducer composition includes a mixture of phosphoglycerides, vegetable oil, non-aromatic solvent, polycyclic aromatic hydrocarbon, and stabilizer.

Coating composition comprising a fatty-acid starch ester

Abraham; T., *et al.*, Cargill, Inc., US10053593, August 21, 2018

A starch-based release coating composition and methods of making the same.

Membrane-based washing and deacidification of oils

Kocherginksy, N., *et al.*, US10065132, September 4, 2018

Membrane-based method of washing and deacidification of oils, wherein a stream of oil is conveyed from an oil reservoir along one side of porous hydrophobic membrane, and washing aqueous solution is conveyed along another side of this membrane. The membranes form hollow fibers, and their total surface area and porosity are large enough for efficient removal of fatty acids, water, ions, and hydrophilic organic impurities from oil. Membrane pore size is small enough, so that hydrodynamic mixing of oil and aqueous solution does not take place. Additional stabilization of oil/water meniscus in the pores is achieved by transmembrane pressure difference.

Carrier comprising non-neutralized tocopheryl phosphate

Libinaki, R., Phosphagenics Ltd., US10071030, September 11, 2018

The present invention relates to a carrier for the delivery of a nutraceutical or cosmeceutical active comprising non-neutralized tocopheryl phosphate and a hydrophobic vehicle. The present invention also relates to a formulation comprising the carrier and a nutraceutical or cosmeceutical active.

Processes for producing hydrocarbons from a renewable feedstock

Ellig, D., *et al.*, UOP LLC, US10065903, September 18, 2018

Processes for the production of linear alkylbenzenes from a renewable feedstock. Prior to converting the side chains of the glycerides and free fatty acids of the feedstock into hydrocarbons, the feedstock is separated into a stream rich in C.sub.10 and C.sub.14 free fatty acids glycerides having C.sub.10 and C.sub.14 fatty acid side chains and at least one, preferably two, other glyceride streams. The stream rich in glycerides having C.sub.10 and C.sub.14 fatty acid side chains can be converted via deoxygenation into a stream rich in C.sub.9 to C.sub.14 hydrocarbons while the other glyceride streams can be used as vegetable oil. A C.sub.10 to C.sub.13 hydrocarbon fraction from the stream rich in C.sub.9 to C.sub.14 hydrocarbons may be dehydrogenated to form olefins which may be reacted with benzene to form linear alkylbenzenes. The linear alkylbenzenes may be used to produce surfactants.

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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As of September 19, 2018

Special issue of *JSD* highlights recent work in Gemini surfactants

Gemini surfactants, an exciting new class of surfactants comprised of two surfactant molecules connected at or near the headgroup with a spacer, exhibit greater surface activity than conventional single-chain surfactants due to increased hydrophobic interaction with water. Their critical micelle concentration (CMC) and interfacial tension are typically much lower than those of conventional surfactants, and they show unique rheological properties and form worm-like micelles and vesicles at low concentration in water.

Now, you can find recent work on new Gemini surfactants in a special collection of papers assembled by the *Journal of Surfactants and Detergents* (*JSD*). Online journal access to the "Gemini Surfactants Virtual Issue" is available to all AOCS members on their "My Account" page. Log in at aocs.org to read any or all of the following papers.

Recent advances in the synthesis of sulfonate Gemini surfactants
Zhou, M., *et al.*, *JSD 21*: 443–453, 2018

Rheology of quaternary ammonium Gemini surfactant solutions: effects of surfactant concentration and counterions
Yang, X., *et al.*, *JSD 21*: 467–474, 2018

Synthesis, characterization, and applications of some new trimeric-type cationic surfactants
El-Said, W.A., *et al.*, *JSD 21*: 343–353, 2018

Aggregation-enhanced emission of fluorescent-Gemini surfactants with high photostability for cell-membrane imaging
Peng, J., *et al.*, *JSD 21*: 433–440, 2018

From left to right: *JSD* Editor-in-Chief George A. Smith; senior associate editors Nancy A. Falk and Carlos Rodriguez-Abreu.

Antimicrobial and cytotoxic properties of bisquaternary ammonium bromides of different spacer length
Ludzik, K., *et al.*, *JSD 21*: 91–99, 2018

Progress in the synthesis of zwitterionic Gemini surfactants
Zhou, M., *et al.*, *JSD 20*: 1243–1254, 2017

Synthesis and performance evaluation of CO_2/N_2 switchable tertiary amine Gemini surfactant
Zhou, M., *et al.*, *JSD 20*: 1483–1489, 2017

Novel ester-linked anionic Gemini surfactant: synthesis, surface-active properties and antimicrobial study
Ahire, M.B., *et al.*, *JSD 20*: 789–797, 2017

Adsorption and micellization of Gemini surfactants with diethylammonium headgroups: effect of the spacer rigidity
Liu, Z., et al., *JSD 20*: 765–773, 2017

Synthesis and evaluation of novel amido-amine cationic Gemini surfactants containing flexible and rigid spacers
Hussain, S.M.S., *et al.*, *JSD 20*: 777–788, 2017

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A Review of Gemini surfactants: potential application in enhanced oil recovery
Kamal, M.S., *et al.*, *JSD 19*: 223–236, 2016

Constructing surfactant systems with the characteristics of Gemini and oligomeric surfactants through noncovalent interaction
Zhu, L., *et al.*, *JSD 19*: 237–247, 2016

Preparation, characterization, and properties of novel cationic Gemini surfactants with rigid amido spacer groups
Xu, D., *et al.*, *JSD 19*: 91–99, 2016

Gemini surfactants with polymethylene spacer: supramolecular structures at solid surface and aggregation in aqueous solution
Pisárčik, M., *et al.*, *JSD 19*: 477–486, 2016

Rheological investigation of wormlike micelles based on Gemini surfactant in EG–water solution
Wei, Y., *et al.*, *JSD 19*: 925–932, 2016

Novel synthesized Gemini surfactant as corrosion inhibitor for carbon steel in HCl solution
Zhang, T., *et al.*, *JSD 18*: 1003–1009, 2015

Properties of a binary system containing anionic and cationic Gemini surfactants
Xu, H., *et al.*, *JSD 18*: 297–302, 2015

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Nanoencapsulation: an efficient technology to boost the antimicrobial potential of plant essential oils in food systems

Prakash, B., *et al.*, *Food Control 89*: 1–11, 2018, https://doi.org/10.1016/j.foodcont.2018.01.018.

Plant essential oils (EOs) possesses remarkable antimicrobial efficacy and therefore have great potential as alternatives to synthetic preservatives. In spite of this potential, they are not widely used by the food industry due to major intrinsic obstacles, such as low water solubility, bioavailability, volatility, and stability within food systems. The application of nanomaterials as carrier agents has recently gained a following in the food industry as a way to improve the efficacy of preservatives at low doses. Nanoemulsions, microemulsions, solid-lipid nanoparticles, and liposomes are some of the currently used encapsulation strategies used to encapsulate plant bioactive compounds. In this review, we explored the potential application of nanoencapsulated plant EOs as a novel source of food preservatives. The prospects, existing limitations, and future research direction for their commercialization are also discussed.

Analytical methods in food additives determination: compounds with functional applications

Martins, F.C.O.L., *et al.*, *Food Chem. 272*: 732–750, 2019, https://doi.org/10.1016/j.foodchem.2018.08.060.

This work describes the 25 classes of food additives that contain about 230 compounds with technological, sensorial, and/or microbiological functionalities. These compounds are added to foods at the processing, packaging, and transport steps, to improve the standard of quality, durability, and stability of the product, and to adjust color, smell, and flavor attributes. This review describes the analytical techniques used to identify and quantify food additives in foodstuffs, highlighting the main characteristics of each method (spectroscopy, chromatography, and electroanalysis) and indicating the advantages and disadvantages typical of each method used. Perspectives of chemical analysis in the food industries are also discussed.

Applications of chia (*Salvia hispanica* L.) in food products

Zettel, V. and B. Hitzmann, *Trends Food Sci. Technol. 80*: 43–50, 2018, https://doi.org/10.1016/j.tifs.2018.07.011.

The outstanding nutritional and technological properties of chia (*Salvia hispanica* L.) have led to innovative applications of chia food products. This overview presents the nutritional and technological properties of chia as well as its technological and innovative uses. Examples of applications in several food and ingredient categories, including baked goods, dairy products, meat and fish products, gluten-free products, functional foods, hydrocolloids, and thickeners, are provided.

Salvia spp. plants-from farm to food applications and phytopharmacotherapy

Sharifi-Rad, M., *et al.*, *Trends Food Sci. Technol. 80*: 242–263, 2018, https://doi.org/10.1016/j.tifs.2018.08.008.

Salvia, one of the largest genera of the family Lamiaceae, is widely distributed throughout the temperate, subtropical, and tropical regions of the world. This review describes the phytochemical composition of essential oils from different *Salvia* species. The biological properties of *Salvia* plants are also discussed.

Screening of antioxidant additives for biodiesel fuels

Varatharajan, K. and D.S. Pushparani, *Renew. Sust. Energ. Rev. 82*: 2017-2028, 2018, http://dx.doi.org/10.1016/j.rser.2017.07.020.

The instability of biodiesel is a longstanding issue that has not yet been satisfactorily resolved. One very promising and cost-effective approach is to add appropriate antioxidants. However, antioxidants perform differently in different biodiesel fuels, and there is no single inhibitor that is suitable for every kind of biodiesel fuel. This article provides an insight into the factors to be considered when selecting antioxidants to improve the storage stability of biodiesel fuels.

Momilactone and related diterpenoids as potential agricultural chemicals

Zhao, M., *et al.*, *J. Agric. Food Chem. 66*: 7859–7872, 2018, https://doi.org/10.1021/acs.jafc.8b02602.

The lack of a fully natural preservative system is a huge gap in the personal care industry. Steroidal lactones and momilactones are plant metabolites expressed in the roots of rice plants. Not much is known about them, but their potential anti-bacterial and anti-fungal properties make them promising new candidates to be screened for possible use in a natural preservative system.

Momilactones are allelochemicals in rice and moss defense. Momilactone-like compounds are therefore considered important secondary metabolites for plant defense. They may serve as promising lead compounds for crop-friendly herbicides as well as antifungal and antibacterial agents. Many of these substances possess potent cytotoxicity properties against cancer cell lines as well. This first review on these versatile molecules focuses on the structure, biological activity, chemical synthesis, and biosynthesis of the naturally occurring momilactone-like molecules reported from 1973 to 2017.

Pectin alleviates high-fat (lard) diet-induced nonalcoholic fatty liver disease in mice: possible role of short-chain fatty acids and gut microbiota regulated by pectin

Li, W., *et al.*, *J. Agric. Food Chem. 66*: 8015–8025, 2018, https://doi.org/10.1021/acs.jafc.8b02979.

This research supports the hypothesis that having a healthy gut microbiota is essential for human health and wellness. It is well understood that fruit and vegetable fiber contribute to stomach and gut health. The data presented here suggest that the soluble fiber pectin helps regulate healthy microbiota and SCFA levels. Hence, it is conceivable that this regulation of microbiota is what drives the health benefits of pectin.

Consumption of pectin contributes to changes in the gut microbiota and the metabolism of short-chain fatty acids (SCFAs). We aimed to investigate the effects of and mechanism by which pectin prevented nonalcoholic fatty liver disease (NAFLD) in mice that were fed a high-fat diet containing 30% lard (HF). HF-fed mice that orally ingested pectin for 8 weeks exhibited improvements in lipid metabolism and decreased oxidative stress and inflammation through a mechanism regulated by the mitogen-activated protein kinase pathway. Pectin dose dependently generated an increase in acetic acid (from 566.4 ± 26.6 to 694.6 ± 35.9 μmol/mL, $p < 0.05$) and propionic acid (from 474.1 ± 84.3 to 887.0 ± 184.7 μmol/mL, $p < 0.05$) contents and significantly increased the relative abundance of *Bacteroides* (from 0.27% to 11.6%), *Parabacteroides* (from 3.9‰ to 5.3%), *Olsenella* (from 2.9‰ to 1.3%), and *Bifidobacterium* (from 0.03% to 1.9%) in the gut of HF-fed mice. Intestinal microbiota and SCFAs may thus contribute to the well-established link between pectin consumption and NAFLD.

Interaction of polyphenols with other food components as a means for their neurological health benefits

Granda, H. and Sonia de Pascual-Teresa, *J. Agric. Food Chem. 66*: 8224–8230, 2018, https://doi.org/10.1021/acs.jafc.8b02839.

This work attempts to understand the mechanistic path by which the polyphenols in fruit, food, and vegetables deliver health

benefits. Polyphenols are extensively studied for their anti-oxidant and anti-inflammation properties, and the commonality of their natural expression cannot be a coincident. These compounds play a role in conjunction with other factors, and this research tries to tease out some of those factors.

Over the last few years, there has been increasing interest in the possible beneficial effect of polyphenol consumption on neurodegenerative disorders. Since environmental factors have a clear impact on the onset and evolution of neurodegenerative conditions, food arises as a possible factor that might influence this group of pathologies. Polyphenols could affect such pathological processes through direct interaction with redox signaling or inflammatory pathways, but their effects could also be explained by the interaction of dietary polyphenols with micro- or macronutrients that are known to have neurological effects, or by preventing neuronal toxicity through interacting with food contaminants or food-associated toxins.

Roles of spicy foods and their bioactive compounds in management of hypercholesterolemia

Zhao, Y., and Z.-Y. Chen, *J. Agric. Food Chem. 66*: 8662–8671, 2018, https://doi.org/10.1021/acs.jafc.8b02975.

This useful review is a one-stop source of pre-clinical and clinical data on many botanicals. The review focuses on the important role that common culinary spices purportedly play in maintaining healthy levels of plasma components.

Culinary spices have attracted special attention as complementary therapies in the management of hypercholesterolemia. The plasma lipid-lowering activities of garlic, ginger, and turmeric have been well-studied in both humans and animals. Consumption of either 3 g/day of ginger or 2 g/day of curcumin for over 4 weeks effectively reduced blood cholesterol in hypercholesterolemia subjects. In contrast, few clinical studies have looked at the effects of chili and black peppers on blood cholesterol. The present review summarizes the findings of recent studies on the efficacy and mechanism of spicy foods and their primary bioactive components in management of hypercholesterolemia from preclinical studies to clinical trials.

Allithiolanes: nine groups of a newly discovered family of sulfur compounds responsible for the bitter off-taste of processed onion

Kubec, R., *et al.*, *J. Agric. Food Chem. 66*: 8783–8794, 2018, https://doi.org/10.1021/acs.jafc.8b03118.

Although the health benefits of onions and their seeds have been professed for years, the known components of onions do not satisfactorily explain some of these claims. Perhaps with more time and focus, we can identify some additional components of onions that are difficult to detect due to volatility or expression levels. Here is an addition to the existing knowledge of onion chemical composition which may help to answer one or two clinical observations.

The compounds responsible for the bitter off-taste of processed onion (*Allium cepa*) were studied. Using a series of sensory-guided HPLC fractionations, the existence of nine groups of hitherto unknown sulfur compounds has been revealed. On the basis of spectroscopic data (MS, NMR, and IR), it was found that these compounds, trivially named allithiolanes A–I, are members of a large family of structurally closely related derivatives of 3,4-dimethylthiolane S-oxide, with the general formulas of $CxHyO_2S_4$, $CxHyO_3S_5$, and $CxHyO_4S_6$ ($x = 10–18$, $y = 18–30$). The presence of multiple stereoisomers was observed for each group of allithiolanes. Allithiolanes possess an unpleasantly bitter taste with detection thresholds in the range of 15–30 ppm. Formation pathways of these newly discovered sulfur compounds were proposed.

Lentil (*Lens culinaris* Medikus) diet affects the gut microbiome and obesity markers in rat

Siva, N., *et al.*, *J. Agric. Food Chem. 66*: 8805–8813, 2018, https://doi.org/10.1021/acs.jafc.8b03254.

Lentils have historically been an important and essential part of the diet for almost a third of the world's population, but we are just now beginning to understand the enormous health benefits arising from them. Even though this is an animal study, the findings fit with the preponderance of epidemiological data on use of lentils.

Lentil, a moderate-energy high-protein pulse crop, provides significant amounts of essential nutrients for healthy living. The objective of this study was to determine if a lentil-based diet affects food and energy intake, body weight, percent body fat, liver weight, and body plasma triacylglycerols (TGs) as well as the composition of fecal microbiota in rats. A total of 36 Sprague–Dawley rats were treated with either a standard diet, a 3.5% high-amylose corn starch diet, or a 70.8% red lentil diet for 6 weeks. By week 6, rats fed the lentil diet had significantly lower mean body weight (443 ± 47 g/rat) than those fed the control (511 ± 51 g/rat) or corn (502 ± 38 g/rat) diets. Further, mean percent body fat and TG concentration were lower, and lean body mass was higher in rats fed the lentil diet than those fed the corn diet. Fecal abundance of *Actinobacteria* and *Bacteriodetes* were greater in rats fed the lentil or corn starch diets than those fed the control diet. Fecal abundance of Firmicutes, a bacterial phylum comprising multiple pathogenic species, decreased in rats fed the lentil and high-amylose corn starch diets vs the control diet. The lentil-based diet decreased body weight, percent body fat, and plasma triacylglycerols in rats and suppressed intestinal colonization by pathogens.

Thermally processed diet greatly affects profiles of amino acids rather than fatty acids in the muscle of carnivorous *Silurus meridionalis*

Zhang, Z., *et al.*, *Food Chem. 256*: 244–251, 2018, https://doi.org/10.1016/j.foodchem.2018.02.066.

This is the first study to evaluate the effects of thermally processed feed on fish. Would thermally processed food have similar effects in humans? If so, what are the implications for human health?

This study aimed to evaluate the effects of thermally processed diet (TD) on the muscle nutritional values of southern catfish in two experiments (named E1 and E2). Compared to non-thermally processed diet (ND), TD did not significantly affect proximate composition of southern catfish, but increased moisture content and decreased protein content in E1. Meanwhile, it had no effect on overall fatty acid profiles of the catfish rich in PUFA. Southern catfish had high proportions of indispensable amino acids (IAA, 44.6–46.4% of total fatty acids), with the highest contents of lysine (1551–1808 mg/100 g wet weight muscle). However, TD altered profiles of the IAA, particularly decreased 68.5% and 68.4% of methionine, and 9.5% and 10.7% of lysine in E1 and E2, respectively. Conversely, it increased 45.4% and 83.4% of dispensable fatty acid proline. These results suggest TD could affect the nutritional quality of protein rather than fat in farmed fish.

Countercurrent chromatographic isolation and purification of 11′-alpha-tocomonoenol from the vitamin E extract of palm oil

Müller, M., *et al.*, *Food Chem.* *256*: 327–332, 2018, https://doi.org/10.1016/j.foodchem.2018.02.133.

A new vitamin E homologue, alpha-tocomonoenol was detected in palm oil, but has not yet been isolated in large amounts or with high purity. Here we present an easy and fast method to isolate alpha-tocomonoenol from vitamin E-rich nutrient capsules with countercurrent chromatography (CCC). With the solvent system n-hexane–benzotrifluoride–acetonitrile (10:3.5:6.5, v/v/v) about 30 mg alpha-tocomonoenol with a purity of 75% could be enriched in one step from 1 g crude sample. Column chromatography with 20% deactivated silica gel and n-hexane–ethyl acetate (95:5, v/v) was performed to gain 5.6 mg alpha-tocomonoenol with a purity of 99.5% according to GC/MS. Structural verification by 1H NMR spectroscopy verified that the double bond was located in 11′-position (11′-alpha-tocomonoenol). The trace impurity detected in the isolate was identified to be 12′-alpha-tocomonoenol, a compound previously detected in marine samples.

Simultaneous extraction of hydrophobic and hydrophilic bioactive compounds from ginger (*Zingiber officinale* Roscoe)

Kou, X., *et al.*, *Food Chem.* *257*: 223–229, 2018, https://doi.org/10.1016/j.foodchem.2018.02.125.

This extraction method enables natural product chemists to harvest the full chemical diversity of bioactive compounds in one process.

Ginger is a commonly used spice around the world. Its bioactive compounds contain hydrophobic gingerols and hydrophilic polysaccharides. Huge physiochemical differences between these compounds and the thermal instability of gingerols impede their fast and effective extraction using conventional methods. In this research, ionic liquid-based ultrasonic-assisted extraction (ILUAE) was applied to simultaneously extract gingerols and polysaccharides from ginger. Parameters influencing the recovery of gingerols were ionic liquid type, ionic liquid concentration, solid/liquid ratio, ultrasonic power, extraction temperature, and extraction time. Compared with traditional methods, LUAE significantly increased the yield of total gingerols and shortened the extraction time. Meanwhile, ginger polysaccharides recovery reached up to 92.82% with ILUAE. Our results indicated that ILUAE has a remarkable capacity to extract gingerols and ginger polysaccharides in one step. Therefore, ILUAE represents a promising technology for simultaneous extraction of hydrophilic and hydrophobic bioactive compounds from plant materials.

Immunomodulatory acidic polysaccharides from *Zizyphus jujuba* cv. Huizao: insights into their chemical characteristics and modes of action

Zou, M., *et al.*, *Food Chem.* *258*: 35–42, 2018, https://doi.org/10.1016/j.foodchem.2018.03.052.

The health benefits of jujube dates documented in traditional literature cannot be explained with the help of known chemistry from this plant. The data reported here point to the utility of polysaccharides as contributing molecules, which could explain some of the benefits.

Chinese jujube is commonly used in folklore medicine. This study aimed to examine the *in vivo* immunomodulatory activity of two acidic polysaccharides, HP1 and HP2, extracted and purified from *Zizyphus jujuba* cv. Huizao (which remains extensively unexplored). HP1 and HP2 had the same monosaccharide species and manganese contents, but differed in their molar rhamnose, arabinose, mannose, glucose, galactose, and uronic acid contents (7.32 and 35.9%, as galacturonic acid), Mw (68.7 and 111 kDa, respectively), and contents of K, Cr, Cu, Zn, Pb, and Ca. Both HP1 and HP2 could significantly ($P < 0.05$) increase spleen and thymus indices, promote serum hemolysin formation, enhance the phagocytic activity of macrophages and inhibit footpad edema of mice, with HP2 likely being a more consistent and potent immunomodulator. This study clearly demonstrates the potential of *Z. jujuba* cv. Huizao polysaccharides as immunomodulators and their associated chemical characteristics and working mechanisms.

Progress in the genetic engineering of cereals to produce essential polyunsaturated fatty acids

Kraic, J., *et al.*, *J. Biotechnol.* *284*: 115–122, 2018, https://doi.org/10.1016/j.jbiotec.2018.08.009.

Essential polyunsaturated fatty acids (PUFA) with more than two double bonds and carbon chain lengths of 18–22 must be taken in the diet to prevent diseases and imbalances caused by their deficiency. Terrestrial sources are limited to only a few plant species. Their large-scale cultivation is not possible, and their seed oil cannot be produced effectively. The complete biosynthetic pathway of fatty acids is known in organisms, including plants. After the first gene-encoding the enzyme catalyzing the initial steps of PUFA bio-





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g. Copies not Distributed *(See Instructions to Publishers #4 (page #3))*		181	187
h. Total *(Sum of 15f and g)*		2847	3036
i. Percent Paid *(15c divided by 15f times 100)*		91.11	92.24

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synthesis (ω-3 desaturase, Δ6-desaturase) were isolated, isolation of other genes encoding relevant enzymes of the PUFA pathway from different donor organisms followed. Genetic transformations of model plants by the desaturase- and elongase-encoding genes opened the way for the genetic engineering of oilseed crop species. Some of the developed transgenic plants produced PUFAs, including eicosapentaenoic and docosahexaenoic acids. Seed oils extracted from them were similar to fish oil. Tools of synthetic biology can be applied in modifications of the PUFA pathway and also in overcoming of limitations when the gene and its expression product are absent in the pathway. Such progress in cereals (barley, wheat, maize) has been made only recently, when the first successful modifications of the ω-3 and ω-6 PUFA pathways succeeded. This review focuses on genetic modifications of the PUFA biosynthetic pathway in cereals in relation to the status reached in model plants and oilseed crops.

Design and synthesis of natural product-inspired libraries based on the three-dimensional (3D) cedrane scaffold: toward the exploration of 3D biological space

Tajabadi, F.M., *et al.*, *J. Med. Chem. 61*: 6609–6628, 2018, https://doi.org/10.1021/acs.jmedchem.8b00194.

A chemoinformatic method was developed to extract nonflat scaffolds embedded in natural products within the *Dictionary of Natural Products* (*DNP*). The cedrane scaffold was then chosen as an example of a nonflat scaffold that directs substituents in three-dimensional (3D) space. A cedrane scaffold that has three orthogonal handles to allow generation of 1D, 2D, and 3D libraries was synthesized on a large scale. These libraries would cover more than 50% of the natural diversity of natural products with an embedded cedrane scaffold. Synthesis of three focused natural product-like libraries based on the 3D cedrane scaffold was achieved. A phenotypic assay was used to test the biological profile of synthesized compounds against normal and Parkinson's patient-derived cells. The cytological profiles of the synthesized analogues based on the cedrane scaffold revealed that this 3D scaffold, prevalidated by nature, can interact with biological systems as it displayed various effects against normal and Parkinson's patient-derived cell lines.