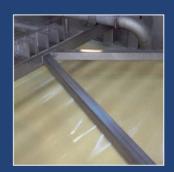




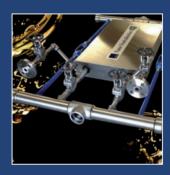
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Using direct solid phase extraction to analyze persistent organic pollutants in oily food samples

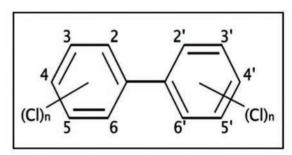
Katherine K. Stenerson

Persistent organic pollutants (POPs) are compounds not easily degraded by chemical, biological, or photolytic processes. Therefore, they persist in the environment for long periods of time. POPs encompass harmful man-made compound classes such as dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides, and polyaromatic hydrocarbons (PAHs). Many of these compounds are lipophilic, and accumulate in the fatty tissues of living organisms. The

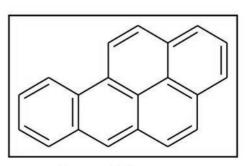
concentrations of POPs increase as they are passed from one organism to another while moving up the food chain (1). Consequently, these compounds can end up in animal-based foods consumed by humans, such as fish, meat, and milk. In the case of plant-based foods such as edible oils, environmental exposure during growing, storage, and/or production can result in POP contamination.

Concern over exposure to these compounds has led to regulation by some countries limiting levels of certain POPs in foodstuffs. For example, maximum PAH levels in edible oils are designated under European Union (EU) Commission Regulation No. 835/2011, and in edible vegetable oils under the National Standard of the People's Republic of China (2,3).

- Many persistent organic pollutants (POPs) such as dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides, and polyaromatic hydrocarbons (PAHs), are lipophilic and can consequently end up in vegetable oils and animal-based foods.
- Conventional analysis of POPs in oily food samples involves laborious extraction and cleanup techniques such as liquid/liquid extraction, column chromatography, and gel permeation chromatography.
- The following article describes a new direct solid phase extraction method that can extract nonpolar contaminants from oily samples easily, quickly, and with good recoveries and reproducibility.



General structure for a PCB



Benzo[a]pyrene



Experimental details

Samples of soybean and fish oils (cod liver) were purchased locally. Soybean oil was spiked at 10 ng/g with the mixture of PAHs listed in Table 1 (page 488).

These PAHs are included on the US EPA Target Compound List (6). Fish oil was spiked at 10 ng/g with the PCB congeners listed in Table 2 (page 489).

These 19 PCB congeners range from mono- to decachlorinated, and include the 12 coplanar species designated by the World Health Organization (WHO) as "dioxin-like" (7). Samples were extracted using the EZ-POP NP cartridge, following the procedures outlined in Table 3 (page 489).

Undiluted oil sample was weighed directly onto the EZ-POP NP cartridge. Figure 1 (page 488) illustrates the cartridge in use during the elution step with acetonitrile.

CONTINUED ON NEXT PAGE

TABLE 1. Acquisition and detection parameters for the selected PAHs analyzed by HPLC-FLD.

Peak #	Compound	t _R (min)	Excitation / Emission wavelengths (nm)
1	Naphthalene	13.08	225/320
2	Acenaphthene	15.38	225/320
3	Fluorene	15.67	225/320
4	Phenanthrene	16.54	250/368
5	Anthracene	17.31	250/368
6	Fluoranthene	18.17	237/440
7	Pyrene	18.88	237/440
8	Benzo[a]anthracene	20.56	265/380
9	Chrysene	20.99	265/380
10	Benzo[b]fluoranthene	22.43	280/420
11	Benzo[k]fluoranthene	23.08	280/420
12	Benzo[a]pyrene	24.03	280/420
13	Dibenzo[a,h]anthracene	24.90	280/420
14	Benzo[g,h,i]perylene	26.65	300/466
15	Indeno[1,2,3-cd]pyrene	27.04	300/466
16	Benzo[b]chrysene (IS)	28.11	300/466

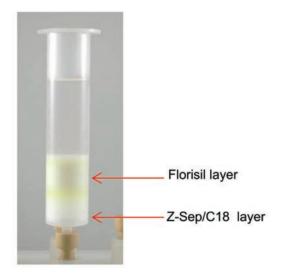


FIG. 1. Dual layer SPE cartridge in use with edible oil sample; extraction of PAHs. The dual layer SPE cartridge contains Florisil (top bed) and a mixture of C18 and zirconia coated silica, commercially available as "Z-Sep" (bottom bed). This combination of sorbents provides multiple mechanisms for retaining fats; adsorption of polar analytes in the case of Florisil, hydrophobic interaction for the C18, and Lewis acid/base interactions for the Z-Sep. Together, these interactions can retain different constituents of fats such as fatty acids and acylglycerols (mono, di, and tri).

For the analysis of PAHs in soybean oil, an internal standard, benzo[b]chrysene, was added directly to the cartridge after sample loading. Spiked soybean oil samples were analyzed in triplicate, and duplicate samples of the unspiked oil were analyzed for comparison. After elution, the soybean oil eluents were concentrated to a final volume of 0.5 mL. Analysis of the extracts was done by high-performance liquid chromatography (HPLC) using a 25 cm x 4.6 mm ID, 5 µm particle size Supelcosil[™] LC-PAH column with a water/acetonitrile gradient. The gradient started at 40% acetonitrile, holding 5 min, then increased to 100% acetonitrile over 15 min, holding at 100% for 12 min. The injection volume was 20 µL, and the column temperature was held at 30 °C. Detection was done by fluorescence, with emission and excitation wavelengths programmed for optimum sensitivity, as described in Table 1.

For the analysis of PCBs in fish oil, no internal standard was used. Replicates of spiked samples (n=6) were prepared, along with triplicate samples of unspiked fish oil. After elution with acetonitrile, the eluents

were extracted with 20 mL of hexane, and concentrated to 1 mL. A second cleanup using silica gel SPE was then done using a 1 mL/300 mg Supelclean LC-Si SPE cartridge, following the procedure in Table 3. The final hexane extracts were concentrated to 1 mL for analysis. Analysis of the fish oil extracts was done by gas chromatography (GC) with an electron capture detector (ECD) using a 20 m x 0.18 mm ID, 0.18 µm SLB*-5ms capillary column. The GC oven conditions were: 75 °C, hold for 1 min, ramp at 12 °C/min to 340 °C and hold for 20 min. The injection port temperature was 250°C, and detector temperature was 340°C. A 1 µL sample injection was done in splitless mode, with the splitter opening at 0.75 min. The carrier gas was hydrogen, used at a 1.2 mL/min constant flow rate.

Quantitation of both PAHs and PCBs was done against 5-point calibration curves prepared in the same solvent as the final sample extracts. Responses of the PAHs were calculated in each sample relative to the internal standard. PCBs were quantitated directly from the calibration curve using absolute response.

PAHs in soybean oil

The extract generated from soybean oil using the EZ-POP NP method was fairly clean of background (Fig. 2, page 491). One problematic matrix peak was present, and it coeluted with fluorene, preventing quantitation of this compound in the spiked samples. All other PAHs were detected free of background. The average recover-

ies obtained for the 3 spiked replicates of soybean oil are presented in Table 4 (page 490).

Detectable levels of several PAHs were present in the unspiked oil, and these values were subtracted from amounts determined in the spikes prior to calculation of recovery. Recoveries of >85% were obtained for all PAHs except naphthalene. Low naphthalene recovery was most likely due to evaporative loss during the concentration step. Reproducibility of the spiked replicates, calculated as percent relative standard deviation (%RSD), was <20% for a majority of the analytes. The higher % RSD values for naphthalene and acenaphthene were due to low values obtained for one or more of the spiked samples. This was, again, most likely due to evaporative loss during concentration.

PCBs in fish oil

The same EZ-POP NP procedure used for the soybean oil was repeated with the fish oil; however the resulting extract had very high background. In the spiked samples, only two PCB congeners could be detected free of matrix (Figure 3, page 491).

For this reason, a secondary cleanup was done with a small silica gel cartridge. This resulted in a very clean background with all congeners detected free of matrix by GC-ECD (Figure 4, page 491).

Three of the target PCB congeners were detected in the unspiked fish oil (Table 5, page 490), however none of these were coplanar, or "dioxin-like." These blank levels were then subtracted from the spiked samples prior to calculating recoveries. The average recoveries obtained for the set of replicates are summarized in Figure 4. Recoveries were between 75 and 120% for all congeners except no. 209. The recovery of no. 209, or decachorobiphenyl, was lower due to its stronger retention on C18 relative to the other less hydrophobic congeners. Reproducibility, calculated as percent relative standard deviation, is indicated in Figure 4 as error bars. The method yielded good reproducibility, with RSD values < 20% for all analytes, and < 10% for 17 of the 19 congeners.

A new SPE method has been presented for the extraction of nonpolar contaminants from oily samples. The method

TABLE 2. Detection parameters for the selected PCB congeners analyzed by GC-ECD.

Peak #	Congener#	IUPAC name	t _{R (} min
1	28	2,4,4'-Trichlorobiphenyl	11.01
2	52	2,2',5,5'-Tetrachlorobiphenyl	11.57
3	101	2,2',4,5,5'-Pentachlorobiphenyl	12.92
4	81	3,4,4',5-Tetrachlorobiphenyl	13.33
5	77	3,3',4,4'-Tetrachlorobiphenyl	13.49
6	123	2',3,4,4',5-Pentachlorobiphenyl	13.81
7	118	2,3',4,4',5-Pentachlorobiphenyl	13.87
8	114	2,3,4,4',5-Pentachlorobipheny	14.01
9	153	2,2',4,4',5,5'-Hexachlorobiphenyl	14.19
10	105	2,3,3',4,4'-Pentachlorobiphenyl	14.24
11	138	2,2',3,4,4',5'-Hexachlorobiphenyl	14.58
12	126	3,3',4,4',5-Pentachlorobiphenyl	14.74
13	167	2,3',4,4',5,5'-Hexachlorobipheny	15.02
14	156	2,3,3',4,4',5-Hexachlorobiphenyl	15.34
15	157	2,3,3',4,4',5'-Hexachlorobiphenyl	15.40
16	180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	15.57
17	169	3,3',4,4',5,5'-Hexachlorobiphenyl	15.89
18	189	2,3,3',4,4',5,5'-Heptachlorobiphenyl	16.41
19	209	Decachlorobiphenyl	17.79

TABLE 3. SPE procedures, soybean and fish oils.

	EZ-POP NP (soybean and fish oils)	Silica gel (fish oil only)
Condition	10 mL acetone,	4 mL acetone, 4 mL hexane
Dry	10 min, -15" Hg	10 min, -15" Hg (after acetone only)
Sample Load	0.5 mL, accurately weighed	1 mL hexane extract
Elution	15 mL acetonitrile	5 mL hexane

TABLE 4. PAHs recovered from soybean oil, unspiked and spiked at 10 ng/g.

	Blank soybean oil		Spiked s	Avg. % Rec. (less blank)		
	Avg. n=2 % RPD°		Avg. <i>n</i> =3	%RSD ^b		
Naphthalene	12.75	6%	13.22	24%	5%	
Acenaphthene	2.98	13%	13.33	28%	103%	
Fluorene			matrix interfe	rence		
Phenanthrene	8.46	10%	17.36	17%	89%	
Anthracene			10.45	18%	105%	
Fluoranthene	2.22	14%	14.54	6%	123%	
Pyrene	0.64	5%	13.71	6%	131%	
Benzo[a]anthracene			13.14	7%	128%	
Chrysene			13.34	6%	133%	
Benzo[b]fluoranthene	0.46	7%	12.97	8%	125%	
Benzo[k]fluoranthene	0.06	28%	12.59	7%	125%	
Benzo[a]pyrene	0.33	19%	11.60	9%	113%	
Dibenzo[a,h]anthracene			10.97	7%	108%	
Benzo[g,h,i]perylene			9.48	21%	93%	
Indeno[1,2,3-cd]pyrene			10.03	14%	100%	

^a %RPD: percent reproducibility

TABLE 5. PCBs detected in unspiked fish oil sample (average *n*=3).

PCB congener	Concentration (ng/g)	% RSD, n=3		
153	16	7%		
138	18	23%		
180	8	9%		

uses a dual layer SPE cartridge containing Florisil (top bed) and a mixture of C18 and zirconia coated silica, commercially available as "Z-Sep" (bottom bed). This combination of sorbents provides multiple mechanisms for retaining fats; adsorption of polar analytes in the case of Florisil, hydrophobic interaction for the C18, and Lewis acid/base interactions for the Z-Sep. Together, these interactions can retain different constituents of fats such as fatty acids and acylglycerols (mono, di and tri). The EZ-POP NP SPE cartridge has been used in the analysis of PAHs from soybean oil, in a direct method which was easy and quick, and yielded good recoveries and reproducibilities for spiked replicates. For animal-derived fats such as fish oil, a secondary cleanup using a small silica gel SPE cartridge provided an extract clean enough for the GC-ECD analysis of PCB congeners. Recoveries of PCB congeners from spiked cod liver oil were >75% with RSD values of <20% for spiked replicates.

^b %RSD: % relative standard deviation

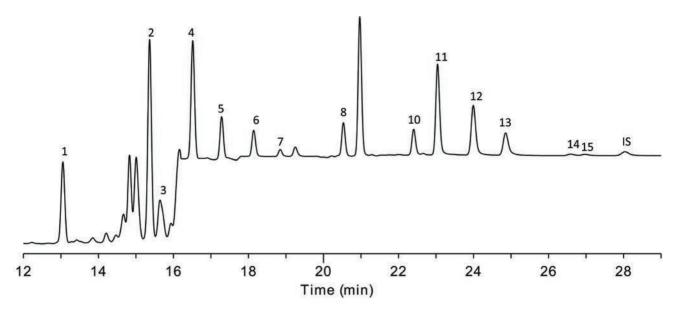


FIG. 2. HPLC-FLD analysis of PAHs in soybean oil spiked at 10 ng/g. Identities of PAH peaks: (1) naphthalene; (2) acenaphthene; (3) fluorene + matrix peak; (4) phenanthrene; (5) anthracene; (6) fluoranthene; (7) pyrene; (8) benzo(a)anthracene; (9) chrysene; (10) benzo(b)fluoranthene; (11) benzo(k)fluoranthene; (12) benzo(a)pyrene; (13) dibenzo(a,h)anthracene; (14) benzo(g,h,i)peryelene; (15) indeno(1,2,3-cd)pyrene; (16) benzo(b)chrysene (I.S.)

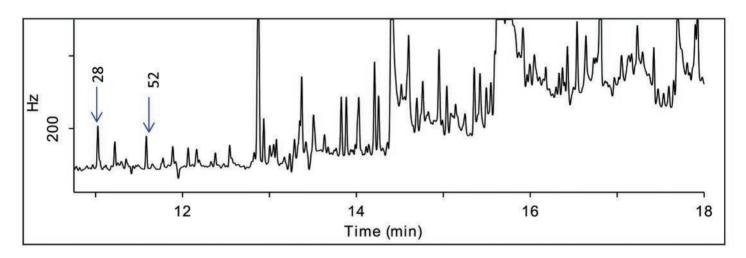


FIG. 3. GC-ECD analysis of spiked fish oil extract prior to secondary silica gel cleanup. Two PCB congeners (indicated) were detected free of background

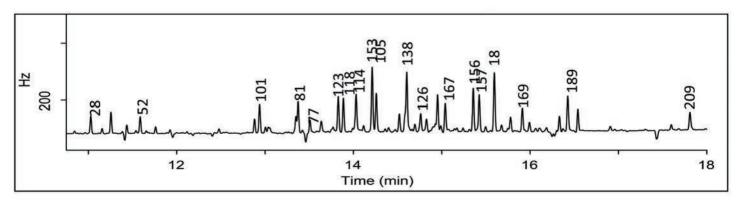


FIG. 4. GC-ECD analysis of spiked fish oil extract (10 ng/g) after secondary silica gel cleanup. PCB congeners are indicated congener number.

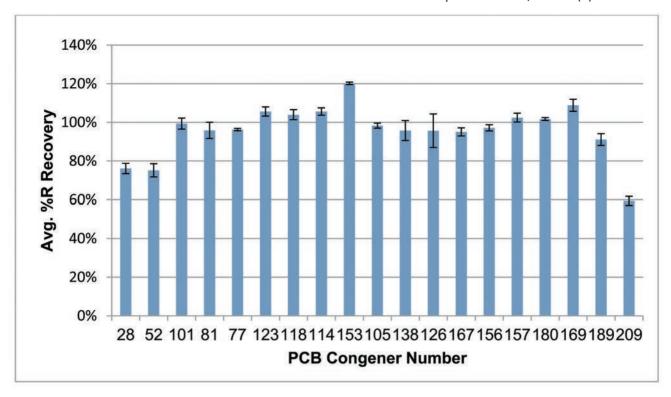


FIG. 5. Average PCB recoveries from spiked fish oil (10 ng/g) after blank subtraction (n=6). The error bars indicate the %RSD for spiked replicates.

The EZ-POP NP cartridge is a new sample preparation tool for the extraction of nonpolar contaminants from oily and high fat samples. Sample preparation methodologies using EZ-POP NP are simple and effective, and do not require the use of chlorinated or other highly toxic solvents. The resulting extracts can be analyzed by HPLC or GC; and in the case of the later technique, background in many cases should be low enough for analysis on a single quadrupole mass spectral detector. In addition to the applications presented here, EZ-POP NP has been utilized successfully in the GC-MS analysis of PAHs in olive oil, canola oil, and butter; and in the GC-ECD analysis of PCBs in soybean oil (8,9). Future sample preparation work using the EZ-POP NP will investigate its utility in analysis of edible oils and oily food samples for other classes of nonpolar contaminants such as organochlorine pesticides.

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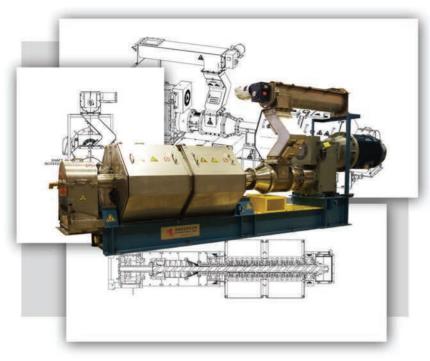
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$$R_{1} = \text{fatty alkyl chain}$$

$$R_{2} = \text{fatty alkyl chain}$$

$$R_{1} = \text{fatty alkyl chain}$$

$$R_{2} = \text{fatty alkyl chain}$$

$$R_{3} = \text{fatty alkyl chain}$$

$$R_{4} = \text{fatty alkyl chain}$$

$$R_{1} = \text{fatty alkyl chain}$$

$$R_{2} = \text{fatty alkyl chain}$$

$$R_{3} = \text{fatty alkyl chain}$$

$$R_{4} = \text{fatty alkyl chain}$$

$$R_{5} = \text{fatty alkyl chain}$$

$$R_{1} = \text{fatty alkyl chain}$$

$$R_{2} = \text{fatty alkyl chain}$$

$$R_{3} = \text{fatty alkyl chain}$$

$$R_{4} = \text{fatty alkyl chain}$$

FIG. 1. Chemical structures of LP (low polar) and HP (high polar) polyamides

Bio-based polyamide gellants for novel product forms and functionality

- Many materials thicken water-based systems, but relatively few are capable of gelling oil-based liquids. This article describes a series of bio-based polyamide gellants that allows product formulators to create novel gelled or thickened materials in both oil-based and aqueous systems.
- The polyamides can produce a range of rheology modification, from thickened flowable formulations to clear firm gels, depending on the concentration of polymer. The gels are reversible, melting at 60–70°C.
- The addition of reactive end-groups to the polyamides results in liquid materials that can be cross-linked at room temperature, leading to clear permanent gels. These stable gels allow controlled fragrance release for air care applications.

B. Scott Jaynes, Hannah Bofinger, and Joe Scheblein

A wide range of polymeric thickening materials currently exist for aqueous systems, including polyacrylic and polyacrylamide based polymers; carboxymethylcellulose derivatives; gelatins; and polysaccharides, such as alginates, carrageenan, xanthan gum, and guar. In contrast relatively few materials are capable of gelling oil-based liquids. Our research group at Croda, Inc. recently introduced a series of partially bio-based polyamide thickeners and gellants that can be selected to reversibly gel both aqueous and



organic/oil-based systems. These polyamide thickeners are chemically stable across a wide range of pH. In this article, we also describe a second family of polyamide gellants with reactive terminal groups that can be cured with a cross-linking material to generate permanent, durable and chemically stable gels with solvents and oils.

A VERSATILE POLYAMIDE **GELLANT SYSTEM**

Croda's polyamide thickeners and gellants are produced from tall oil fatty acid feedstocks that are dimerized over a catalyst to generate a liquid mixture of "dimer acids." These dimer acids are then polymerized with diamines to generate polyamides, which are terminated with either ester or amide functional groups, as shown in Fig. 1. The terminal groups on the low-polarity (LP) series of polyamides are fatty alkyl chains that give the materials hydrophobic character so that they are compatible with oil and solvent-based formulations. In contrast, the high-polarity (HP) series of thickeners is terminated with polyether functionality and short alkyl chain capping groups, giving the polymers hydrophilic character so that they are suitable for thickening high-polar solvents and aqueous formulations. These materials are covered by a series of US patents (US 6,268,466; US 6,399,713; US 6,469,131; US 6,503,077; US 6,503,522)

The LP and HP polyamides are prepared as 100% solid beads that can be dissolved in an appropriate solvent by heating to ~95°C for several minutes until the solids dissolve. Upon cooling, the hydrogen bonding network between the polymer chains re-forms, causing the solvent to be trapped in a gel matrix. In the case of gels formed with the low-polar LP materials, the solvent/oil can be visualized as sitting in the hydrophobic pockets of the dimer portion of the polyamide matrix. In the case of more polar solvents and aqueous systems, the solvent will reside in the regions of the polyether portions of the polymer matrix It should be noted that the melting point of gelled materials is lower than that of the 100% polymer solid, usually between 60-70°C. As a result, the LP and HP gels are thermally reversible by heating to this temperature range.

The ability to tune the polyamide polarity by the appropriate choice of terminal and linking group chemistries provides a remarkably versatile family of thickeners and gellants. As discussed above, the LP series is capable of gelling non-polar materials including mineral oil, ester solvents, fragrance oils, and other organic liquids. In contrast, the HP series is capable of gelling polar materials including polar

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Gelled fragrance oil



- 25% Polyamide LP
- 65% Ester solvent
- 10% fragrance

Gel will melt at ~60°C

Gelled surfactant



- 25% Polyamide HP
- 75% Nonionic surfactant (LAE)
- Water soluble gel

Gel will melt at ~60°C

Figure 2. Examples of gelled fragrance (on left) and gelled nonionic surfactant (on right)

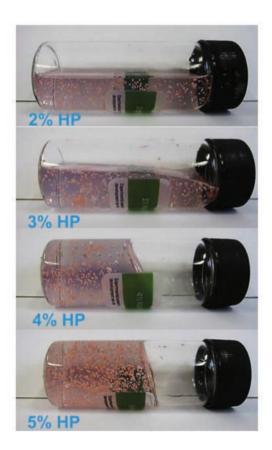


FIG. 3. Behavior of hard-surface cleaning formulation containing 2%, 3%, 4%, and 5% HP polyamide (with suspended beads)

solvents, dipropylene and larger glycols, aqueous systems, and even nonionic surfactants. Examples of gels prepared with both LP and HP materials are shown in Fig. 2. In the first case, clear gel was prepared from an ester solvent with 10% fragrance oil, resulting in a clear firm fragrance gel. Fragrance oils can also be used as the sole solvent, resulting in fragrance concentrations of up to 80% in the final gels. In the second case, a pure nonionic surfactant (alcohol ethoxylate) was gelled with the HP material, giving a clear gelled surfactant suitable for various gelled cleaning applications such as toilet rim blocks.

RHEOLOGY MODIFICATION FOR AQUEOUS CLEANING SOLUTIONS

While the polyamides provide firm gels when added at 20–30% of a formulation, reduced levels of the polymer can generate lower levels of rheology modification that can provide important performance benefits for both aqueous and non-aqueous flowable systems. As an example, the HP polyamide was added at various levels to a hard-surface cleaning solution containing 2% nonionic surfactant in water. The polyamide was added at 2%, 3%, 4%, and 5% to the cleaning solution to illustrate the range of rheology modification possible with this system. As shown in Fig. 3, addition of 2% resulted in a slightly thickened, but readily flowable system. Addition of 3% gave a thicker, but still flowable system, while addition of 4% or 5% resulted in soft gels that did not flow. The thickened solutions exhibit shear thinning rheology, which allows the products to be easily sprayed through a nozzle, yet regain their gel like properties once applied to the surface. This "spray-and-stick" capability

is important in hard-surface cleaning applications where the cleaning solution will cling to vertical surfaces and allow the chemical cleaning action to be more effective before running off the surface.

An example of the performance of a cleaning solution on a vertical surface with and without the HP polyamide is shown in Fig. 4. Additionally, the shear thinning rheology of these dilute polyamide solutions provides excellent suspension properties for particles, beads, encapsulated actives, or any other solid particles that need to be suspended in a formulation.

ODOR-ABSORBING GEL APPLICATION

The polyamide gellants are chemically robust and well-suited for gelling active components in aqueous or non-aqueous systems. As an example, a series of gels was prepared incorporating an odor absorbing active material from Croda called Forestall® (*N*-ethyl-*N*-soya morpholinium ethosulphate). As the Forestall is a water-soluble cationic material, the gel was prepared in HP polyamide, using a blend of dipropylene glycol and water as a solvent. The formulation and an image of the clear gel (with dye added) are shown in Fig. 5. To prepare the gel, all components were heated to 95°C for five minutes, then the solution was cooled in a suitable mold or container until gelled.

While Forestall was previously shown to be effective at odor absorption and control when used in an aerosol spray application, it was unclear whether the active would be equally effective in a gelled product format. To evaluate its odor-control performance, a panel study was designed with twenty panelists who were asked to evaluate the strength of onion odor in sets of large glass jars. Samples of polyamide HP gel were prepared with and without the Forestall active and placed in the jars. Chopped onion was then added to each jar for 15 min to infuse the jar with onion odor, then the onion was removed. Panelists were asked to evaluate the odor in the jars (one with Forestall, one without) and decide which jar had less odor. Evaluations were made at T = 1, 3, 5, and 24 hr. As shown in Fig. 6 (page

Surfactant Solution 2% HP Solution



FIG. 4. Image of hard surface cleaning solution sprayed on vertical tile, with and without HP polyamide thickener

498), after 1 hour, 17 out of 20 panelists identified the jar containing the Forestall active in the gel as having lower odor than a jar with polyamide HP gel alone. Significantly more panelists chose the Forestall-containing jar at 3 and 5 hours as well. After 24 hours, the odor had dissipated or been completely absorbed, so no difference was discerned. The results indicate that the Forestall odor-absorbing active is highly effective in a gel format, resulting in a convenient and attractive product which can deliver odor absorption and fragrance delivery benefits simultaneously.

CROSS-LINKABLE PERMANENT GELS

Replacement of the terminal ester or amide groups in the LP series with reactive amine- or hydroxyl-terminated groups, results in a liquid material that can be cross-linked at room temperature to yield permanent, clear, and flexible gels. Two different variations of these "polyamide IM" materials are utilized,

CONTINUED ON NEXT PAGE

Ingredient	Grams in Formulation	
Water	15	pll
Dipropylene Glycol	52.5	J. St.
Forestall (30% Active)	7.5	and the state of t
Polyamide HP	20	
Fragrance (optional)	5	

FIG. 5. Formulation and image of Forestall odor absorber polyamide HP gel formulation.

Jars observed by panelists with less onion odor

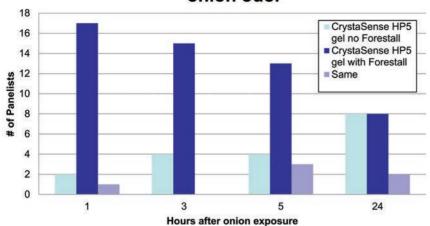


FIG. 6. Odor panel test results showing lower onion odor in jars containing forestall odor absorbing active

Component	%	460	- 1	-				
Polyamide IM700	19.5			1.0	-			
Polyamide IM800	6.5	60						
Alkyl benzoate	35	No.		. \$	-			
Fragrance	35			1				
Curing agent	4							
70	nilla vender 6 8 10	12 14 16 Day		0 22	24	26	28	30

FIG. 7. Formulation of cross-linked fragrance gels using polyamide IM materials, and fragrance release from gels over 30 days

one designed to cure rapidly (IM700) and the other to cure more slowly (IM800). By combining these two liquid materials in an appropriate ratio with an isocyanurate cross-linker and a solvent, the room temperature curing rate can be tuned to whatever time is desirable. The total suggested polymer concentration for these gels is 26% with 4% added cross-linker, while the remaining 70% of the formulation can be a combination of fragrance and/or solvent. As

a result, flexible, non-tacky gelled fragrance blocks can be produced with fragrance levels up to 70% in a durable clear gel format.

As an example, gelled fragrance blocks containing 35% vanilla or lavender fragrance blocks were generated at room temperature using the formulation shown in Fig. 7. The clear flexible gels were subsequently monitored for weight loss of fragrance over 30 days, showing the gradual release of fragrance from the gels. (The rate of fragrance loss will depend on the type of fragrance, concentration and shape of the gel.) The controlled release of fragrance over an extended time period illustrates how these materials can be used to generate attractive, durable, and chemically robust gels for air care application.

Croda's polyamide gellants offer a versatile range of bio-based products for rheology control in a wide variety of consumer products. Clear, firm, meltable gels can be generated from both non-polar and polar liquids, including solvents, fragrances, oils, surfactants, and aqueous formulations. Thickened flowable formulations can be generated with lower levels of polyamide, resulting in sprayable products with high-suspension capability. By adding reactive functional groups to the polyamides, cross-linkable materials are generated and can be cured at room temperature to yield gelled fragrances with active levels of up to 70%. These permanent gels are ideally suited for controlled fragrance release in a variety of air care product applications.

Scott Jaynes is the R&D applications manager for home care at Croda, Inc., Edison, New Jersey, USA, where he is responsible for new product development and technical service for home care technologies in North America. Prior to joining Croda, Jaynes held positions at Unilever, Ciba Specialty Chemicals, and BASF. He can be contacted at scott.jaynes@croda.com.

Hannah Bofinger is a graduate trainee at Croda, Inc. In her role in Home Care Applications R&D, she was responsible for assisting in formulation development and applications testing. Prior to working at Croda, she previously held positions at Cognis/BASF, McNeil Consumer Healthcare, and International Flavors and Fragrances.

Joe is a lead applications scientist in Home Care R&D at Croda, Inc. He has over 25 years of industrial experience in both raw materials and

consumer products, including product development and formulation of a wide variety of products designed for the household/industrial and institutional markets.

BRIEFS

Fields of canola are being grown 25 kilometers from the Fukushima Daiichi nuclear plant in Japan in an effort to remove radioactive cesium from the soil, according to *The Wall Street Journal* newspaper (see http://tinyurl.com/Canola-Cesium). The objective is to create a chain of decontamination and power generation by using a biomass power station to make electricity from methane given off by fermenting canola. The project has tripled in size since it began with 4.7 hectares in the fall of 2013.

ПТ

Solazyme is pursuing regulatory approvals for a new co-product for animal feed, according to a report by SeekingAlpha.com (see http://tinyurl. com/Sola-Co). The company, known for tailored algal oils, has not generally answered the question regarding what happens to the algal biomass after extraction. Now, research published in the Journal of Animal Science (http://dx.doi.org/10.1016/j. watres.2010.08.037, 2015) examines the results of testing algal biomass as a high-energy feedstuff and protein supplement for various livestock. Commercial sales could begin as soon as 2016, SeekingAlpha.com said. The article also suggested other possible Solazyme co-products, based on its patent applications, including fire logs, adsorbents, paper products, thermoplastics and thermosets, and wood plastic and thermoplastic composites.

IN MEMORIAM

Michael (Mike) Pulliam, a long-term AOCS member, died on June 20, 2015. He was 62.

Pulliam received a bachelor's degree in chemical engineering from the University of Missouri-Rolla. He was employed by C&T Quincy, LLC, before joining ADM, which purchased C&T in 1998. Mr. Pulliam was general manager of ADM Quincy refinery at the time of his death.

Mr. Pulliam's service to AOCS was extensive, including service on the Budget Committee, Books & Special Publications Committee, Financial Activities Coordinating Committee, and Investments Committee.

NEWS & NOTEWORTHY



Regulatory update: TSCA, FSMA, PHOs

It has been a busy time for US regulators, with varying degrees of action on an alphabet soup of regulations, including TSCA, FSMA, and the FDA's final determination on the GRAS status of partially hydrogenated vegetable oils.

Beginning with the legislative effort with the least amount of activity to report, reform of the primary piece of US legislation covering chemicals policy—the Toxic Substances Control Act of 1976—continued as *Inform* went to press.

The House of Representatives passed its TSCA Modernization Act (HR 2576) by a vote of 398–1, on June 23, 2015. Meanwhile, the Senate's alternative— the Frank R. Lautenberg Chemical Safety for the 21st Century Act (S 697)—had not yet been brought to the floor, even as Senator Barbara Boxer (Democrat-California) urged the Senate to adopt the House-passed legislation instead.

Also in late June, the chief sponsors of the House and Senate bills indicated that an informal conference to negotiate differences between the two ahead of presenting the legislation to a formal Conference might improve the chances of passage.

FINAL FSMA COUNTDOWN

A US Food and Drug Administration (FDA) spokesperson told *Inform* in July that the

agency "is committed to meeting the courtmandated deadlines for the implementation of the FSMA rules," including the August 30 deadline for the release of the two final rules concerning preventive controls for human and animal food.

Once the final rules are published, the compliance deadlines for both rules, for businesses with 500+ employees, will be one year after publication. Businesses with fewer than 500 employees must be in compliance by two years after publication. Those with less than \$2.5 million in annual sales will have three years after final publication to comply.

FSMA, which stands for the FDA Food Safety Modernization Act of 2010, aims to help FDA prevent food safety incidents before they occur rather than simply reacting to incidents after they happen. As such, the Act gives far-reaching new regulatory and enforcement powers to FDA and will affect virtually every partner in the food and feed supply chains.

Under the regulations, FDA requires manufacturers to gather up to two years of verification data of their suppliers' observance of the regulations, which must be accessible to FDA within 24 hours of a request for data. CEOs are ultimately responsible for verifying that their company's suppliers are in compliance under FSMA, which ups the compliance ante significantly.

How FDA will fund its new responsibilities under FSMA has been a continuing

CONTINUED ON PAGE 501

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question. In June 2015, the US House Appropriations Committee approved a \$41.5 million increase in funding for FSMA for fiscal year 2016. That amount, however, is less than half the amount requested. Even the full \$109.5 million request for 2016, though, is less than the \$580 million the Congressional Budget Office estimated FSMA would cost over a five-year period.

Also of note: FDA has published a proposed rule and a draft guidance document to support a new program under FSMA that will help eligible foreign entities demonstrate that imported food meets US food safety requirements. Audits and certifications for eligible foreign entities under this program will be used by importers applying for expedited review through the Voluntary Qualified Importer Program, and may also be required by FDA as a condition of granting imported food admission into the United States when certain food safety risks have been identified. Visit http://tinyurl.com/FSMA-PR to read the proposed rule and/or comment by October 7, 2015; visit http://tinyurl.com/FSMA-DG for the draft guidance.

In related news, four European voluntary standard-setting groups have agreed to harmonize requirements for purchasing feed ingredients and services at the national, European, and international levels. The four groups are the Agricultural Industries Confederation (UK), GMP+International (Netherlands), OVOCOM (Belgium), and QS Qualität und Sicherheit (Germany). The harmonized requirements should be available before the end of 2016. The groups noted in a news release that their aim is to ensure that every company in the supply chain will promote feed safety by applying the best practices and demonstrate this via third-party certification.

DETERMINATION ON PHOS

Perhaps the biggest regulatory story of the previous quarter is the announcement by the FDA on June 16, 2015, that it has revoked the GRAS (generally recognized as safe) status of partially hydrogenated oils (PHOs) and changed the regulatory status of PHOs to that of a food additive. Food manufacturers have until June 18, 2018, to either remove PHOs from their products or submit a food additive petition to FDA.

"Based on the available scientific evidence and the findings of expert scientific panels, [FDA] has made a final determination that there is no longer a consensus among qualified experts that PHOs, which are the primary dietary source of industrially produced trans fatty acids (TFAs), are generally recognized as safe for any use in human food," the agency said in its declaratory notice (see http://tinyurl.com/FDA-PHO-2015).

As food additives, PHOs are now subject to \$409 of the Federal Food, Drug, and Cosmetic Act. This means that any use of PHOs in foods will require the submission of a food additive petition for FDA's review and approval. The Grocery Manufacturers Association (GMA; Washington, DC, USA) has submitted a petition on behalf of its members that GMA said in a statement "show[s] that the presence of trans fat from the proposed low-level uses of PHOs is as safe as the naturally occurring trans fat present in the normal diet. Food and beverage companies have already voluntarily lowered the amount of trans fat added to food products by more than 86% and will continue lowering PHO use in foods."

As *Inform* went to press, the petition was being reviewed by FDA to make sure it includes all the necessary information for the agency to conduct its evaluation, according to GMA spokesperson Brian Kennedy. Kennedy also said that GMA will not release any details

AOCS methods for trans fatty acid analysis

The US Food and Drug Administration and international industry experts have recognized and utilized AOCS methods to determine trans fatty acid profiles in foods.

• AOCS Official Method Ce 1h-05 (http://tinyurl.com/trans-05)—"Determination of *cis-, trans-*, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-Ruminant Animal Oils and Fats by Capillary GLC"

The fatty acid methyl esters of vegetable and nonruminant animal fat and oil samples are separated according to chain length, degree of unsaturation, and geometry and position of double bonds by capillary GLC with a highly polar stationary phase.

• AOCS Official Method Ce 1j-07 (http://tinyurl.com/trans-07)—"Determination of cis-, trans-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC"

The fatty acid methyl esters of extracted fat samples are separated according to chain length, degree of unsaturation, and geometry and position of double bonds by capillary GLC with a highly polar stationary phase.

• AOCS Official Method Ce 2b-11 (http://tinyurl.com/trans-b-11)—"Direct Methylation of Lipids in Foods by Alkali Hydrolysis"

Fatty acid methyl esters directly from food matrices are prepared with simultaneous alkali hydrolysis and methylation without prior digestion. Incorporation of triacylglycerol standards allows the quantification of total fat and fatty acids using Theoretical Correction Factors and Empirical Correction Factors.

• AOCS Official Method Ce 2c-11 (http://tinyurl.com/trans-c-11)—"Direct Methylation of Lipids in Foods by Acid-Alkali Hydrolysis"

Fatty acid methyl esters directly from food matrices are prepared by *in situ* acid digestion followed by alkali hydrolysis and methylation.

about the petition until the FDA accepts it and makes the "several-hundred-page document" available for public review.

Although the vast majority of PHOs have been removed from the food supply, a number of niche applications remain. Ingredients with low levels of TFAs include processing aids, pan-release agents, anti-caking agents, emulsifiers, spices, and encapsulates for flavor agents and color additives. Food products that might still contain TFAs include certain baked goods, canned cake icing, microwave popcorn, chewing gum, and frozen pizza.

 $Ingredients\ synthesized\ using\ PHOs\ but\ not\ containing\ PHOs\ in$ the final product are a grey area, according to attorney\ Robert\ A.\ Hahn

CONTINUED ON NEXT PAGE

of the Olsson Frank Weeda Terman Matz PC law firm in Washington, DC, USA. Hahn presented his analysis of the determination during a live webinar on June 25 hosted by AOCS. A recording of the webinar is available online at http://tinyurl.com/AOCS-Webinar.

"FDA has intimated that if such ingredients are GRAS based on self-determination, a re-evaluation of the self-determination may be in order," noted Hahn

The legal impact of the determination, he added, is that as of June 18, 2018, PHOs will be a food additive; therefore, their use in human food will be deemed as adulteration. The determination also left the door open for state legislation and litigation by saying there is no express preemption of current state or local laws banning or limiting the use of PHOs. The agency declined to take a position on implied preemption, said Hahn, but did state that it does not believe that state and/or local laws banning PHOs would conflict with federal law or frustrate federal objectives.

"This signals that state and local governments are free to enact their own laws and plaintiffs are free to sue under state laws," he said.

Hahn found it "unlikely" that FDA will approve any food additive petitions for PHOs since the determination found that there is a linear relationship between TFA intake and the risk of heart disease. Thus, FDA has determined that there is no safe level of PHOs in the food supply. That determination, however, is based on very little data concerning low levels of intake, he noted.

"It will be difficult for FDA to back down from its position," he said.

According to Hahn, the declaratory order does not affect:

- Fully hydrogenated oils (those with an iodine value of 4 or less),
- Refined oils,

- Naturally occurring trans fat in meat and dairy,
- Conjugated linoleic acid,
- Partially hydrogenated methyl ester of rosin,
- Ingredients made using PHOs as raw materials (where the ingredient does not contain PHOs), and
- PHOs used in animal feed.

FDA will not begin enforcement until after June 18, 2018, said Hahn, after which point the agency could conceivably, during FSMA inspections, see if processors' and manufacturers' preventive control plans indicate they are monitoring ingredients that in the past would have contained PHOs to ensure they no longer contain PHOs.

Hahn mentioned that an ILSI [International Life Sciences Institute] North America Task Force is working to close some of the data gaps concerning industrially produced TFAs and the risks associated with different levels of intake. Three manuscripts representing three phases of work are in the final stages before submission to a peer-reviewed journal for publication, an ILSI spokesperson said in an email. The objective of Phase 1 was to develop an evidence map to better characterize the existing literature regarding intervention trials on TFAs. The objective of Phase 2 was to establish a biological understanding of a threshold (mode of action determination) and conduct a meta-regression based on the relevant epidemiological studies to improve the overall understanding of the shape of the dose-response curve. In Phase 3, based on the meta-regression, ILSI aims to identify a point of departure and considerations for extrapolation to lower doses of TFAs. As work is published, it will be available on the ILSI website at http://tinyurl.com/ILSI-PHOs.

Despite the final determination, it appears that much remains to be determined. \blacksquare

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The Schroepfer Medal

Originated by colleagues of George Schroepfer, this award recognizes a scientist who has made significant and distinguished advances in the steroid field. The work may represent a single major achievement or an accumulation of data.

> Nature of the Award: An honorarium and a medal.

Deadline: October 15





ACI/NBB Glycerine Innovation

The Industrial Oil Products Division initiated this award to recognize outstanding achievement for research in new applications for glycerine with particular emphasis on commercial viability.

Nature of the Award: \$5,000 and a plaque provided by the American Cleaning Institute and the National Biodiesel Board.

Deadline: November 1

Samuel Rosen Memorial

Milton Rosen and the Surfactants and Detergents Division initiated this award to recognize a surfactant chemist for significant advancement or application of surfactant chemistry principles.

Nature of the Award: \$2,000 and a plaque.

Deadline: November 1

Herbert J. Dutton

The Analytical Division initiated this award to recognize an individual who has made significant contributions to the analysis of fats and oils and related products or whose work has resulted in major advances in the understanding of processes utilized in the fats and oils industry.

Nature of the Award: \$1,000, a travel-and-expense allowance, and a plaque.

Deadline: November 1

Timothy L. Mounts

The Edible Applications Technology Division initiated this award to recognize research relating to the science and technology of edible oils or derivatives in food products, which may be basic or applied in nature.

Nature of the Award: \$750 and a plaque provided by Bunge North America.

Deadline: November 1

Edible Applications Technology Outstanding Achievement

This award recognizes a scientist, technologist, or leader who has made significant contributions to the Division's field of interest, or made contributions to the advancement of edible oils.

Nature of the Award: \$500 and a plague.

Deadline: November 1

MINATIONS

Ralph Holman Lifetime Achievement

The Health and Nutrition Division established this award to annually recognize an individual who has made significant contributions to the Division's field of interest, or whose work has resulted in major advances in health and nutrition.

Nature of the Award: \$500, a travel-and-expense allowance, and a signed orchid print.

Deadline: November 1



The award recognizes and honors outstanding, meritorious service to the oilseed processing industry.

> Nature of the Award: Travel-and-expense allowance and a certificate.

> > Deadline: December 1

Surfactants and Detergents Distinguished Service

The award recognizes outstanding, commendable service to the surfactants, detergents and soaps industry.

> Nature of the Award: A plaque. Deadline: December 1



Alton E. Bailey

This award is supported by the USA Section and recognizes research and/or service in the fields of fats and oils and related disciplines.

Nature of the Award: \$750 and a plaque.

Deadline: November 1



Thomas H. Smouse Fellowship

This award was established by the Archer Daniels Midland Foundation and the family and friends of Thomas H. Smouse. The purpose of this graduate fellowship is to encourage and support outstanding research by recognizing a graduate student pursuing an M.S. and/or Ph.D. degree in a field of study consistent with the areas of interest of AOCS.

Nature of the Award: The Fellowship level is up to \$15,000 (\$10,000 Fellowship, \$5,000 for travel and research expenditures related to the student's graduate program).

Deadline: February 1

Ralph H. Potts Memorial **Fellowship**

This award recognizes a graduate student working in the field of chemistry of fats and oils and their derivatives. Qualifying research will involve fatty acids and their derivatives, such as long-chain alcohols, amines, and other nitrogen compounds.

Nature of the Award: \$2,000, a plague, and traveland-expense allowance. The award is supported by AkzoNobel, Inc.

Deadline: October 15

Honored Student

This award recognizes graduate students in any area of fats and lipids. To receive the award, a candidate must remain a registered graduate student and must not have received a graduate degree or have begun career employment prior to the Society's Annual Meeting.

Nature of the Award: Travel-and-expense allowance and a certificate.

Deadline: October 15

Hans Kaunitz

This award is supported by the USA Section and encourages studies in the sciences relating to fats, oils, and detergent technology. This award is open to graduate students within the geographical boundaries of the USA

Nature of the Award: \$1,000, a travel-and-expense allowance, and a certificate.

Deadline: October 15

AOCS Division Awards

These awards recognize students at any institution of higher learning, who are studying and doing research towards an advanced degree in fats, oils, proteins, lipids, surfactants, detergents, and related materials.

for Students

The following student awards are currently being offered by these AOCS Divisions:

Analytical Division Student Award

Biotechnology Student Excellence Award

Edible Applications Technology Division Student Award

Health and Nutrition Division Student Excellence Award

Industrial Oil Products Division Student Award

Lipid Oxidation and Quality Division Student Poster

Processing Division Student Excellence Award

Protein and Co-Products Division Student Poster

Surfactants and Detergents Division Student Travel

Award Nature of the Award: Awards can consist of

\$100 to \$1,000 and a certificate.

Deadline: Varies from October 15 to January 15

See website.



The award recipient must agree to attend the AOCS Annual Meeting & Expo and present an award address. The 107th AOCS Annual Meeting & Expo will be held in Salt Lake City, Utah, USA from May 1–4, 2016.

AOCS Awards contact > awards@aocs.org • www.aocs.org/awards



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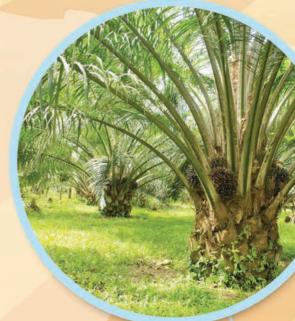
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Matt Rudolph of the Roundtable on Sustainable Biomaterials (Geneva, Switzerland) presented an overview of 10 emerging feedstocks for biofuels at the recent Advanced Bioeconomy Feedstocks Conference (see http://tinyurl.com/RBS-ABFC). Rudolph covered jatropha, carinata, *Gliricidia sepium*, pongamia, switchgrass, giant miscanthus, starchy wastewater, industrial waste gases, Solaris tobacco seeds, and the macauba palm tree (*Acrocomia* sp.). More highlights from the meeting are available from *Biofuels Digest* at www. biofuelsdigest.com.

Indonesia's Sungai Budi will invest \$22.4 million in 2015 to expand its palm oil plantations and \$15 million to build a biodiesel plant. The biodiesel plant could eventually produce up to 35% of the company's revenues, according to the *Jakarta Globe* newspaper.

BiofuelsDigest.com also reports that 661.5 million liters of biodiesel were sold in Brazil at the National Petroleum Agency's most recent auction on June 17 and 18, 2015. Thirty-four producers offered nearly 825 million liters of biodiesel at the auction, with 97.05% accredited by Brazil's Social Fuel sustainability program.

The Port of Seattle wants the Seattle-Tacoma (USA) International Airport to be the first US airport to offer biofuels to all airlines. Seattle Port Commission members told the *Puget Sound Business Journal* newspaper that they are "working to create financial incentives like those being implemented in Oslo and Amsterdam that would make aviation biofuels more affordable." The group plans to release a framework for how to proceed by the end of the year.

India's Ministry of Shipping has decided to meet 20% of its diesel requirements with biodiesel at 12 major ports, according to the *Hindu Business Line* newspaper. Emami Agrotech Ltd.'s 300-metric-ton-per-day biodiesel refinery in Haldia will supply biodiesel to that city's port. Further, Emami is working with the government to boost the use of biodiesel in ports, railways, and across the transport sector, according to the report.

ENERGY



Mechanism and structure of two key enzymes isolated from yeast

Researchers at the UK's Manchester Institute of Biotechnology have identified the mechanism and structure of two key enzymes isolated from yeast molds that together provide a new, cleaner route to the production of hydrocarbons.

Published in *Nature*, the research offers the possibility of replacing the need for petroleum in current industrial processes with a greener and more sustainable natural process (http://dx.doi.org/10.1038/nature14559, 2015).

Lead investigator David Leys explains the importance of the work: "Whilst the direct production of fuel compounds by living organisms is an attractive process, it is currently not one that is well understood. And although the

potential for large-scale biological hydrocarbon production exists, in its current form it would not support industrial application, let alone provide a valid alternative to fossil fuels."

Leys and his team investigated in detail the mechanism whereby common yeast mold can produce kerosene-like odors when grown on food containing the preservative sorbic acid. They found that these organisms use a previously unknown modified form of vitamin B2 to support the production of volatile hydrocarbons that caused the kerosene smell. Their findings also revealed the same process is used to support synthesis of vitamin Q10 (ubiquinone).

Using the Diamond synchrotron source at Harwell, they were able to provide atomic-level insights into this biocatalytic process, and reveal it shares similarities with procedures commonly used in chemical synthesis but previously thought not to occur in nature.

"Now that we understand how yeast and other microbes can produce very modest

CONTINUED ON NEXT PAGE

amounts of fuel-like compounds through this modified vitamin B2-dependent process," says Leys, "we are in a much better position to try to improve the yield and nature of the compounds produced."

In the study, the researchers focused on the production of α -olefins. These are a high-value, industrially crucial intermediate class of hydrocarbons that are key chemical intermediates in a variety of applications, such as flexible and rigid packaging and pipes, synthetic lubricants used in heavy-duty motor and gear oils, surfactants, detergents, and lubricant additives.

"This fundamental research... provides the basis for the development of new applications in biofuel and commodity chemical production," notes Leys. "The insights from this research offer the possibility of circumventing current industrial processes [that] are reliant on scarce natural resources."

The Biotechnology and Biological Sciences Research Council and Shell Chemicals funded the research.

DOE awards \$18 million to algae projects

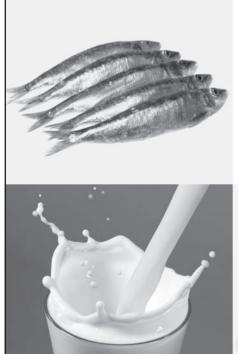
In July 2015, the US Department of Energy (DOE) announced six projects that will receive up to \$18 million in funding to reduce the modeled price of algae-based biofuels to less than \$5 per gasoline gallon equivalent (gge) by 2019. "This funding supports the development of a bioeconomy that can help create green jobs, spur innovation, improve the environment, and achieve national energy security," the DOE said in a news release.

Algal biomass can be converted to advanced biofuels that offer promising alternatives to petroleum-based diesel and jet fuels. Additionally, algae can be used to make a range of other valuable bioproducts, such as industrial chemicals, biobased polymers, and proteins. However, barriers related to algae cultivation, harvesting, and conversion to fuels and products need to be overcome to achieve DOE's target of \$3 per gge for advanced algal biofuels by 2030.

To accomplish this goal, DOE is investing in applied research and development technologies that can achieve higher yields of targeted bioproducts and biofuels from algae—increasing the overall value for algae biomass. The projects selected include:

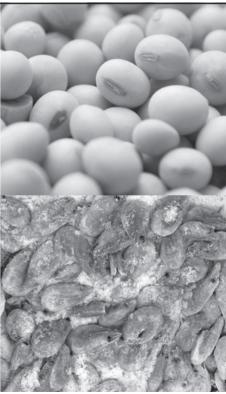
- Producing Algae and Co-Products for Energy—Colorado School of Mines (Golden, Colorado), in collaboration with Los Alamos National Laboratory, Reliance Industries Ltd., and others, will receive up to \$9 million to enhance overall algal biofuels sustainability by maximizing carbon dioxide, nutrient, and water recovery and recycling, as well as bio-power co-generation.
- Marine Algae Industrialization Consortium—Duke University (Durham, North Carolina) will receive up to \$5.2 million to lead a consortium including the University of Hawaii, Cornell University, Cellana, and others to produce protein-based human and poultry nutritional products along with hydrotreated algal oil extract.
- Global Algae Innovations, Inc.—Global Algae Innovations (El Cajon, California) will receive up to \$1 million to increase algal biomass yield by deploying an

CONTINUED ON PAGE 539



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Welcome New Members



AOCS is proud to welcome our newest members*.

*New and reinstated members joined from April 1 through June 30, 2015.

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AOCS is excited to introduce a proficiency testing series for Distillers Dried Grains with Solubles (DDGS) from corn meal. The first year includes 6 samples (2 in September; 2 in December; 2 in March). The analytes of interest are: moisture, crude protein, crude fat, crude fiber, and ash (optional). Results are requested in duplicate for method validation purposes. Laboratories are encouraged to use the listed methods (see below) though alternate methods are acceptable provided a method reference is remitted.

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Moisture: NFTA 2.2.2.5 (105 °C/3 hr)
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BRIEFS

A team of Belgian researchers has bred hybrid yeast strains that work particularly well in chocolate fermentation, according to a recent paper in the journal Applied and Environmental Microbiology (http://doi. org/10.1128/AEM.00133-15, 2015). Normally, the natural microbiota present at a cacao farm ferment the pulp surrounding cacao beans. However, differences in microbes at different farms can cause inconsistencies in the flavor and quality of the resulting chocolate. Kevin J. Verstrepen at the University of Leuven and his colleagues wanted to develop a microbial starter culture that could outcompete wild contaminants and consistently produce high-quality chocolate. So they screened more than 1,000 strains of the yeast Saccharomyces cerevisiae to find strains that could efficiently ferment cacao beans at the high temperatures encountered during fermentation (45-50°C). The researchers then crossed some of the best strains to produce hybrids that performed even better. The team's industrial partner, Barry Callebaut (Zürich, Switzerland), made chocolate from beans fermented by the newly developed yeast strains. In taste tests, consumers preferred this chocolate over chocolate produced by conventional fermentation.

In a recent article in Mayo Clinic Proceedings, researchers led by Edward Archer at the University of Alabama at Birmingham (USA) argue that much of the data underpinning the US Government's 2015 Dietary Guidelines for Americans is scientifically flawed (http://doi.org/10.1016/j. mayocp.2015.04.009, 2015). These data arise mainly from memorybased dietary assessment methods (M-BMs) such as interviews and surveys. Because M-BMs rely on human memory and recall, rather than independent observation, they are too inaccurate and precise, the authors say. Moreover, the majority of the data in question are physiologically implausible—in other words, a person could not survive on what they claim to have eaten and drank, "Our

FOOD, HEALTH & NUTRITION



Dietary seed oil effects on renal oxylipins

Oils such as canola and soybean contain different types of fatty acids with distinct effects on the human body. Many of these effects arise from oxylipins—biologically active molecules produced when enzymes in the body oxidize polyunsaturated fatty acids from the diet. However, the relationship between levels of fatty acids and their oxylipins is not always straightforward, explained Harold Aukema from the University of Manitoba, in Winnipeg, Canada, in a talk at the 106th AOCS Annual Meeting and Industry Showcases, held May 3–6, 2015, in Orlando, Florida, USA.

The two major polyunsaturated fats in dietary oils are linoleic acid (LA; an omega-6 fatty acid) and α -linolenic acid (ALA; an omega-3 fatty acid). Surprisingly, the oxylipins derived from these fatty acids are almost completely unexplored, says Aukema. Enzymes convert LA into arachidonic acid

AOCS ANNUAL MEETING COVERAGE



(AA), which is a precursor of various oxylipins that cause pain and inflammation, such as prostaglandins. However, consuming more LA in the diet does not change the level of AA in the bloodstream, so scientists have assumed that oxylipin levels remain unchanged, as well.

In a 2013 study, Aukema fed obese rats dietary oils (e.g., canola, soy, safflower) with different amounts of LA (http://dx.doi. org/10.3945/jn.113.177360, 2013). For rats fed a high-LA diet, no changes in renal phospholipid AA were observed, but there were substantial changes in some AA and LA oxylipins. Aukema concluded that even though fatty acid levels may not change substantially with different types of oil in the diet, oxylipin levels do.

CONTINUED ON NEXT PAGE

work indicates there is no scientific foundation to past or present US Dietary Guidelines," says Archer, which may explain why nutritional recommendations are constantly changing, he says.

Following a comprehensive review, the European Food Safety Authority (EFSA) has concluded that acrylamide in food is a public health concern (http://doi.org/10.2903/j. efsa.2015.4104). The scientific opinion cites animal studies in which acrylamide and its metabolite glycidamide damage DNA and cause cancer. However, human studies linking dietary acrylamide and cancer are limited and inconclusive. Acrylamide forms when starchy foods are cooked at high temperatures, such as during baking and frying. The food groups contributing most to acrylamide exposure are fried potato products, coffee, biscuits, crackers, and bread.

The other major polyunsaturated fat in dietary oils, ALA, is converted into the beneficial fatty acid docosahexaenoic acid (DHA) within the body. However, this reaction is thought to take place at a very low efficiency, so some dietary guidelines recommend supplementation of DHA at 200-400 mg/day. In a recent study (http://dx.doi.org/10.1016/j.plefa.2014.11.009, 2014), Aukema examined a mouse kidney disease model that shows reduced renal DHA and DHA oxylipin levels. Flax oil, which is rich in ALA, slows disease progression in these mice. So Aukema and his colleagues investigated the effects of dietary flax oil on renal fatty acids and oxylipins. They found that although there was no change in DHA level when the mice were fed flax oil, levels of DHA oxylipins increased. Therefore, dietary flax oil could reduce disease progression by mitigating DHA oxylipin abnormalities. The study also provided evidence that in vivo ALA conversion to DHA occurs in amounts sufficient to restore DHA oxylipin levels. Therefore, oxylipin levels may provide data not available from fatty acid levels.

Fast new method for detecting gutter oils

Gutter oils—oils that are collected and refined from kitchen waste, animal fats, restaurant fryers, and sewers—are becoming a worldwide health risk. Detecting these oils, which are fraudulently labeled as normal edible oils, in a timely manner has proven difficult for regulators. Now researchers have developed a simple new method to quickly authenticate edible oils and detect gutter oils (http://dx.doi.org/10.1016.j.aca.2015.05.013, 2015).

Adulteration of edible oils, for example, diluting a more expensive oil (such as olive) with a cheaper oil (such as canola), has long been a problem in the food industry. But more recently, gutter oils have increased in prevalence, and the toxic and carcinogenic compounds in these oils pose serious risks to human health. In 2013, experts estimated that one-tenth of cooking oils in mainland China were gutter oils, and the problem has since spread to Taiwan, Hong Kong, Singapore, and other countries.

Currently, there is no widely accepted scientific method to quickly and reliably identify gutter oils. Approaches developed thus far involve detecting food residue markers in the oils, such as capsaicinoids from chili peppers, eugenol from seasonings, or undecanoic acid from heated vegetable oils. However, any one marker is unlikely to be found in all gutter oils, and markers can be removed by processing.

Mass spectrometry (MS) is used frequently to analyze edible oils, but existing protocols require labor-intensive and time-consuming sample preparation. Therefore, researchers led by Zhong-Ping Yao at The Hong Kong Polytechnic University, in Hung Hom Kowloon, developed a simplified sample preparation method that allows direct analysis of edible oils by matrix-assisted laser desorption/ionization (MALDI)-MS.

In conventional MALDI-MS, scientists mix the edible oil sample with a "matrix" material that helps the sample become ionized upon irradiation with a laser. A mass spectrometer detects the resulting ions, allowing identification of specific molecules in the oil. In the new method, Yao's team loaded the oil samples directly onto a surface that was pre-coated with the matrix material, and then analyzed the samples by MS. The new protocol saves time because no sample extraction or premixing of matrix and sample solutions is required. Also, the simplified protocol enables automated sample loading and high-throughput analysis. Analyzing one sample takes only minutes, compared with hours for conventional approaches.

The new method easily differentiated between different types of edible oils such as olive, peanut, and canola. The researchers established a preliminary spectral database of 32 edible oils, so that they could determine the authenticity of an edible oil sample by comparing its MALDI-MS spectrum and principal component analysis results with those in the database. Yao's team showed that they could differentiate pure vegetable oils from mixed oils, and they could even determine the compositions of mixed oils. The approach also easily detected gutter oils because their MALDI-MS spectra did not correspond with any of the edible oils in the database.

Yao's team is now working on establishing a more complete spectral library and validating the method for the analysis of other mixed and gutter oil samples. If the method holds up to this additional scrutiny, it could "play an important role in authentication of edible oils," the authors say.

New food thickener with probiotic potential

Microbiologists at Oregon State University (OSU; Corvallis, USA) have discovered and helped patent and commercialize a new food thickener derived from nonpathogenic bacteria, according to a news release from the university dated June 9 (http://tinyurl.com/Ropy352). In addition to thickening fermented dairy products, the bacterial strain that produces the polymer may impart probiotic characteristics.

The biopolymer results from decades of research at OSU. In the 1990s, Janine Trempy and her colleagues discovered a natural bacterial strain, called *Lactococcus lactis* subsp. *cremoris* Ropy352 (Ropy352), that could quickly thicken milk. The team then traced the thickening activity to an exopolysaccharide

composed of glucose and galactose in a molar ratio of 3:2. The researchers found that the novel exopolysaccharide arises from a unique combination of sugar-polymerizing genes, or galactosyltransferases, residing on a plasmid in the bacterium.

In 2007, Trempy and her colleagues obtained a US Patent for the Ropy352 biopolymer. Recently, OSU agreed to issue a nonexclusive license for the Ropy352 technology to an undisclosed global market leader for dairy starter cultures. The biopolymer is available for further licensing through OSU's Office of Commercialization and Corporate Development.

When researchers add Ropy352 or the polymer it produces to liquids such as whole and nonfat milk, lactose-free milk, coconut milk, and rice milk, the polymer makes the liquid very thick and smooth, with a rich, slightly sweet flavor. Initial applications will likely lie in fermented dairy products such as sour cream, yogurt, buttermilk, and cheeses. The thickener may also find uses in cosmetic and personal care products such as creams, lotions, and serums.

"There are actually very few new, nondisease-causing bacterial strains that produce unique polymers with characteristics desirable and safe for food products," says Trempy. Although generally regarded as safe, xanthan gum, a common thickener in the food industry, is produced by the pathogenic bacterium *Xanthomonas campestris*. In contrast, lactic acid bacteria similar to Ropy352 have been used for centuries to make fermented milk products consumed by humans. In addition, some strains of lactic acid bacteria are considered probiotics—microorganisms that provide health benefits when consumed or applied to the skin. Therefore, Ropy352 may serve a dual function in fermented dairy, personal care, or cosmetic products.



Have you ever wondered, as you reached for your bottle of extra virgin olive oil (EVOO), what percentage market share EVOO holds in the global market?

Inform recently posed that question to Paul Miller, president of the Australian Olive Association and advisor to the Olive Oil Commission of California. It just so happens that Miller answered the question during a presentation at a May 27 workshop in San Francisco titled "Defending Your Supply Chain: The Case of Extra Virgin Olive Oil."

Based on Australian olive industry analyses of various data from a number of government and industry sources, Miller says that EVOO holds an estimated 21% market share of the global market, with virgin olive oil accounting for 29% and refined olive oil maintaining a 50% share.





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BRIEFS

ity (EFSA) has issued new guidance on data requirements for genetically modified (GM) plant risk assessefsa.2015.4128, 2015). The document complements existing guidance on specific recommendations on how to notypic and agronomic data for GM plants. Those seeking market approval for a GM plant in the European Union must compare the phenotypic (e.g., plant height and color) and agronomic (e.g., yield and pesticide resistance) characteristics of the GM plant with its non-GM counterpart. The new guidetions for the selection of test materials and sites, the quality and design of field trials, and the selection of specific plant characteristics to assess the plant's biology and behavior.

In a statement released July 2, 2015, the US Office of Science and Technology Policy (OSTP) announced plans to modernize the regulatory system for tory system is governed by the Coordinated Framework for the Regulation of Biotechnology (CF), which was last lish a Biotechnology Working Group that will update the CF to clarify the of the US Department of Agriculture, Environmental Protection Agency, and ulating biotechnology products. The OSTP aims to streamline the regulatory approval process to reduce unnecessary costs and burdens, while protecting health and the environment. Other objectives of the regulatory overhaul include increasing transparency and promoting public confidence in the regulatory process.

New information changes few opinions on genetically modified organisms (GMOs), according to a recent study in the journal *Food Policy* (http://doi. org/10.1016/j.foodpol.2015.04.010, 2015). In the study, Brandon R. McFadden at the University of Flor-CONTINUED ON NEXT PAGE

BIOTECHNOLOGY



Rewilding: the future of organic farming?

For millennia, humans have used selective breeding to produce crop plants with high yields and other desirable properties. However, selecting one beneficial trait can unintentionally sacrifice another; therefore, many of today's crops are more susceptible to diseases, insects, and competition from weeds than their wild ancestors. "Rewilding" involves reintroducing to modern crops lost traits of their ancestors, often through the use of biotechnology. In a recent review in Trends in Plant Science, a team of Danish researchers makes the case that rewilding may particularly benefit a segment of agriculture typically opposed to modern biotechnology techniques—namely, organic farming (Trends Plant Sci., http://dx.doi. org/10.1016/j.tplants.2015.04.011, 2015).

For health and environmental reasons, organic farmers avoid synthetic fertilizers,

herbicides, and pesticides, instead relying on "natural" methods such as composting and insect predators. However, compared with conventional agriculture, organic farming suffers from reduced productivity. "To close the yield gap between organic and conventional farming, organic farming is in need of more robust plants," write the researchers, led by Michael G. Palmgren at the University of Copenhagen, in Denmark. "By bringing back select lost properties of their ancestors, rewilding has the potential to increase genetic diversity and reintroduce wild traits that would benefit organic farming."

Modern biotechnology techniques that can be used for rewilding include cisgenesis, in which a complete gene is transferred from a wild relative into a domesticated crop, and precision breeding, in which a specific mutation at a single nucleotide position is introduced to a gene in the domesticated crop to make it identical to an ancestral gene.

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ida (Gainesville, USA) and Jayson L. Lusk at Oklahoma State University (Stillwater, USA) surveyed 961 people across the United States via the Internet. The researchers determined the participants' beliefs by asking them to respond to statements such as, "Genetically modified crops are safe to eat." Then, the participants were given scientific information that GMOs are safe and asked again about their beliefs. Before they received the information, 32% believed GM foods were safe, 32% were unsure, and 36% did not believe GM foods were safe. After receiving the information, 45% of participants believed that GMOs were safer than they had believed previously, 43% were unswayed by the information, and 12% believed GMOs were less safe than they had believed before receiving the information.

In cisgenesis, a bacterial shuttle, *Agrobacterium*, transfers the ancestral plant gene to the domesticated plant genome. The resulting plant genome may contain some bacterial DNA sequences, although techniques exist to remove these sequences or make them identical to those found in plants. However, because new gene material is inserted, the rewilded plant would be considered a genetically modified organism (GMO) in the European Union. Using cisgenesis, scientists have rewilded potato plants for improved late blight resistance and apple trees for scab resistance.

For precision breeding, also known as genome editing, a DNA-cleaving enzyme cuts DNA at a specific site within the gene of interest, which generates a double-stranded DNA break. The plant cell then repairs the break, in the process introducing a specific mutation that makes the modern crop gene identical to the ancestral gene. Whether rewilded plants generated by precision breeding would be considered GMOs is uncertain, say the authors. GM techniques are used in the

process, but no new gene material is inserted in the plant. Because the techniques used in precision breeding are so new, there are as yet no examples of crops rewilded with this approach.

Palmgren and his colleagues argue that rewilding is compatible with many of the principles of organic farming. For example, rewilded crops could allow organic farmers to avoid fertilizers and pesticides without loss of yield. In addition, rewilded crops would be more "natural" than traditionally bred crops because they more closely resemble their wild ancestors. Yet because surveys indicate that most organic farmers reject GM crops, it is unclear whether they would ultimately accept rewilded crops. Likewise, consumers who currently purchase organic foods are generally against GM foods. However, many consumers who express a preference for organic foods are unwilling to pay premium prices for them. If the cost of organic foods drops as a result of rewilding, more consumers may be willing to purchase them, enlarging the now-small organic market share.

Whether rewilded crops could be labeled as organic is a central issue, and the answer may vary depending on a legislative body's definition of GMO. In the EU, GMOs are defined as organisms arising from the use of specific biotechnology methods. In contrast, the United States and Canada define GMOs as organisms having a new combination of genetic material that could not have occurred naturally. Therefore, rewilded crops produced by precision breeding would likely be considered GMOs in the EU, and thus incompatible with an organic label, but may not be considered GMOs in the USA and Canada. The authors write that the EU may need to rethink its process-based regulatory framework for defining GMOs. "If plants obtained from [rewilding techniques] cannot be distinguished from crops bred by conventional means, they should be exempted from the current GMO legal framework," the authors say.

AOCS MEETING WATCH

October 4–6, 2015. 25th Canadian Conference on Fats and Oils, co-organized by the Canadian section of the American Oil Chemists' Society and the Consortium for Research and Innovation in Industrial Bioprocesses, Delta Hotel Québec, Québec City, Québec, Canada. http://tinyurl.com/CAOCS-meeting

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

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

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

October 5–7, 2016. World Conference on Fabric and Home Care—Singapore 2016, Shangri-La Hotel, Singapore. http://singapore.aocs.org

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

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

New collaboration to advance world's first stress-tolerant soybean

A new collaboration among Bioceres S.A. (Rosario, Argentina), Arcadia Biosciences, Inc. (Davis, California, USA), and Tropical Melhoramento e Genética Ltda. (TMG; Cambé, Brazil) has brought the first stress-tolerant soybean one step closer to commercialization. The collaboration, announced in a joint news release on July 7 (http://tinyurl.com/stresstolerantsoybean), builds upon a previous partnership between Bioceres and Arcadia Biosciences—known as Verdeca LLC—to develop the HB4 stress-tolerance trait.

The HB4 trait makes soybeans stress-tolerant primarily by repressing the ethylene signaling pathway. Ethylene is a gaseous plant hormone that, among other functions, signals plant cells to stop dividing, or become senescent. The transcription factor HB4 (from sunflower) inhibits ethylene-induced senescence, which may allow plants to maintain photosynthesis for longer periods and better survive drought or other stressors. Researchers introduced the sunflower HB4 gene into soybeans via an *Agrobacterium* vector.

The HB4 trait has undergone six seasons of field trials in Argentina and the United States and two years of regulatory field trials. These trials revealed that the HB4 trait improves soybean yield by up to 14% under multiple stress conditions and by about 7% in good conditions. In April 2015, the



HB4 trait received regulatory approval from CONABIA, the National Advisory Commission on Agricultural Biotechnology, in Argentina—the first regulatory approval for a stress-tolerant trait in soybeans. Verdeca is currently seeking approval of the HB4 soybean in Brazil and the United States. According to Verdeca's website (http://www.verdeca.com), the company expects the initial HB4 soybean varieties to become commercially available between 2015 and 2017.

The new collaboration partners Verdeca with TMG, a major Brazilian soybean seed company. "This collaboration leverages Verdeca's trait technology and regulatory expertise with TMG's world-class soybean germplasm, breeding capabilities, and significant market share in the South American soybean seed market," said Eric Rey, president and CEO of

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Arcadia Biosciences. According to the news release, South America is the world's largest exporter of soybeans, and more than 45% of the world's soybeans are grown in Argentina and Brazil



Licensing deal to commercialize super-high-oleic safflower oil

Australia's national science agency, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), has

entered into an exclusive worldwide license with GO Resources to commercialize super-high-oleic safflower oil (SHOSO), according to a July 13 news release on GO's website (http://www.go-resources.com.au). GO Resources (Williamstown, Australia) is a clean technology company that produces renewable, biodegradable raw materials for industrial and oleochemical markets.

As a renewable alternative to petroleum-based raw materials, SHOSO could serve as a raw material for biobased feed-stocks, with applications in lubricant, oleochemical, cosmetic, biofuel, and other industries. Oleic acid, a monounsaturated fatty acid, is traditionally obtained at much lower levels from tallow, olive, palm, and oilseeds.

Super-high-oleic safflower seeds produce very high levels of oleic acid (>92%) in oil extracted from the seed. According to the news release, this level of oleic acid is the highest of any commercially available, plant-derived oil. Olive oil contains 83% oleic acid, and high-oleic soybean oil has about 75% oleic acid. CSIRO developed super-high-oleic safflower using RNA interference to silence genes encoding the enzymes that convert oleic acid into polyunsaturated fatty acids.

"We have proven that we can tailor safflower to produce extremely high levels of oleic acid in the seeds," says Allan Green, research director of CSIRO's Bioproducts Program. "This technology is a great example of how plant oils are uniquely suited for expanded use as industrial raw materials and substitutes for current petrochemicals."

GO Resources anticipates that Australian commercial production of SHOSO will commence in 2018. ■



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Genetic ID was the first commercial laboratory to offer DNA-based testing for GMOs and is accredited to ISO 17025, the leading international standard for testing laboratories, by A2LA.

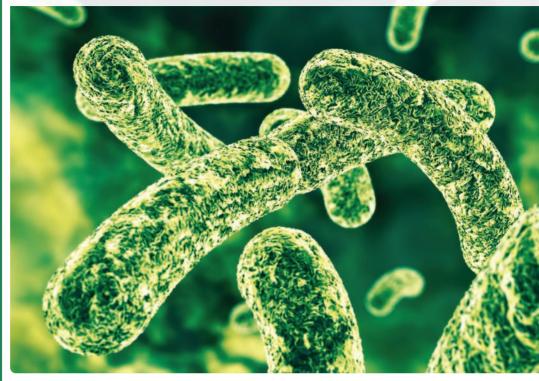
As a benefit of corporate membership, companies are entitled to provide a 250-word profile for inclusion on a space-available basis in Inform magazine. For more information, contact Nicole Philyaw at nicolep@ aocs.org.

Procter & Gamble (P&G; Cincinnati, Ohio, USA) has accepted an offer of \$12.5 billion from beauty products manufacturer Coty, Inc. (New York City, USA), to merge 43 color cosmetics, fragrance, and hair care brands. To save P&G from paying capital-gains taxes, the deal will be structured as a Reverse Morris Trust, in which the beauty brands will be separated from P&G and merged with a Coty subsidiary. Included in the transaction are hair care brands Clairol, Wella, and Nice & Easy; fragrance lines Hugo Boss, Dolce & Gabbana, and Gucci; and color cosmetics lines Max Factor and Cover-Girl. The transaction will mark Coty's first entry into hair care lines and will expand the company's geographical range into Brazil, Japan, and other large beauty markets.

REACH (Registration, Evaluation, Authorisation, and Restriction of CHemicals) may be having the unintended consequence of stifling innovation, according to a survey of 1,600 companies conducted by the European Commission. REACH, which took effect in the European Union (EU) in June 2007, regulates the production and use of chemicals with the goal of protecting human health and the environment. The preliminary results of the survey, announced at the Helsinki Chemicals Forum May 28-29, 2015, indicate that 35% of the companies surveyed say that REACH is negatively affecting their capacity to innovate, with only 10% claiming a positive impact. Almost 40% of chemical manufacturers said that REACH has affected their competitiveness compared to companies outside the EU, with 75% claiming the regulations have weakened their competitiveness. In contrast, 57% of chemical suppliers and almost 45% of end users said that REACH has strengthened their competitive positions.

Declining oil prices will slow growth in the oil field chemicals market for 2015-2019, according to a new global market study by IHS, Inc. (The IHS Chemical 2015 Specialty Chemicals Update Report—Oil Field Chemicals). ■

HOME & PERSONAL CARE



Lactic acid, in combination with surfactants commonly used in cleaning products, kills bacteria and reduces viral infectivity.

Lactic acid, a natural biocide for detergents

As traditional biocides face increasing scrutiny over health and environmental concerns, formulators are searching for green alternatives that are compatible with detergents in cleaning products. Lactic acid, a natural biocide, offers several advantages as an antimicrobial agent for detergent applications, according to Esther Lansdaal of Corbion (Amsterdam, Netherlands) in a talk she presented at the 106th AOCS Annual Meeting and Industry Showcases, held May 3-6, 2015, in Orlando, Florida, USA.

Many traditional biocides such as triclosan are under pressure because of their possible toxicity, contamination of wastewater from consumer use, and propensity for promoting bacterial resistance. An alternative to traditional biocides, lactic acid is an effective natural biocide that works synergistically with other commonly used home care and detergent ingredients, including surfactants.

AOCS ANNUAL MEETING COVERAGE



Lactic acid can kill bacteria by increasing the hydrogen ion concentration within the bacterial cell. An enzyme called ATPase pumps H⁺ back out of the cell, but it requires energy to do so. If the lactic acid concentration is sufficiently high, the bacterium expends all of its energy pumping out H+, eventually killing the microbe. In addition to this antibacterial mechanism, lactic acid can also act as a preservative, inhibiting bacterial growth. Bacteria produce energy by glycolysis, and a high concentration of lactic acid outside the cell blocks this pathway so that bacteria cannot grow and proliferate.

Lactic acid can also inactivate viruses such as influenza. In an acidic environment, the hemagglutinin protein found on the surface of the influenza virus dissociates, rendering the virus unable to bind and infect

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host cells. Similarly, lactic acid inactivates human immunodeficiency virus (HIV). This effect is not solely due to pH, however, because lactic acid inactivated HIV better than acetic acid or water at the same pH. Lactic acid reduced the infectivity of both enveloped and nonenveloped (capsid) viruses, even in the presence of the surfactant sodium lauryl sulfate (SLS).

According to Lansdaal, lactic acid by itself is not as potent as traditional biocides and is only effective at relatively high concentrations (about 10%). However, common detergent ingredients such as surfactants and alcohols increase bacterial membrane permeability, boosting the biocidal efficacy of lactic acid at lower concentrations (1–2%). Anionic surfactants give the best synergistic effects. Lactic acid is compatible but not synergistic with nonionic surfactants, and it is sometimes synergistic and sometimes incompatible with cationic surfactants.

In a sample formulation of a hard surface cleaner, lactic acid in combination with SLS and alkyl polyglycoside surfactants killed bacteria within one minute and fungi within 15 minutes. In a second sample formulation, an antibacterial manual dish liquid containing lactic acid showed 10–15% better performance (number of plates cleaned) than two commercial benchmarks.

Lactic acid is registered for sanitizing and disinfecting cleaning formulations in Europe, the United States, and Canada, and is compliant with various ecolabels. Lactic acid is carbon dioxide-neutral, nontoxic, and readily biodegradable.

Consumer Reports rejects unit-dose liquid detergents

Citing safety concerns, *Consumer Reports* will no longer include unit-dose liquid laundry detergents on its list of recommended laundry detergents, states an article posted on the company's website on July 16 (http://tinyurl.com/unitdose). The article notes that in 2014, poison-control centers in the United States received 11,714 reports of children 5 years old and younger ingesting or inhaling the laundry detergent pods.

Consumer Reports is a US-based magazine and associated website that publishes reviews and comparisons of consumer products. The company bases its reviews on reporting, in-house testing, and consumer surveys. In 2014 the magazine had 8.4 million subscribers and an operating revenue of \$265.8 million, making it an influential source of product information for consumers.

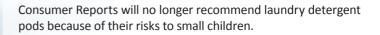
Unit-dose detergents, also called laundry detergent pods, entered the US market in 2012. The pods are convenient and effective for washing clothes; however, their candy-like appearance and scent are enticing to small children. When a child sucks on a pod or bites into one, the liquid detergent that is released can cause serious health problems such as vomiting, lethargy, delirium, and respiratory distress. Since 2013, at least two children have died after ingesting a pod. For reasons even doctors don't yet understand, liquid pods are much more toxic than other types of laundry detergent, such as regular liquid detergent or pods containing powder detergent.

"Given the continued danger, we have made the decision to not include pods on our list of recommended laundry detergents," writes *Consumer Reports*. "And we strongly urge households where children younger than six are ever present to skip

them altogether." The magazine's decision does not apply to laundry or dishwasher pods that contain powder because they have caused fewer and less severe injuries.

As early as September 2012, Consumer Reports urged manufacturers to make unit-dose detergents safer, and many have responded. For example, Proctor & Gamble, the manufacturer of Tide Pods, introduced an opaque container with three safety latches to make the pods more difficult for children to see and access. In addition, the container displays more prominent safety warnings. But according to Consumer Reports, further steps are needed to ensure product safety.

Safety experts from the company have participated in the development of a voluntary safety standard for laundry pods, led by the global standards-writing organization ASTM International. The latest draft of the standard, released in June 2015, calls for several improvements that have already been implemented in Europe, including the addition of a bittering agent to give pods a bad taste; a higher "burst strength" to make them more difficult to bite into; and a slower dissolution rate. In the United States, members of Congress have introduced the Detergent Poisoning and Child Safety Act of 2015, which, if it becomes law, would establish additional safety standards, such



as requiring manufactures to redesign the pods so that they no longer resemble candy.

The American Cleaning Institute (ACI) issued a statement in response to *Consumer Reports*' decision (http://tinyurl.com/ACIpods), noting that unit-dose laundry detergents are used safely in approximately 26 million households. "Educating parents and caregivers is vital to the prevention of any type of injury in the home, and the cleaning products industry will continue to work to help prevent accidental access to these products," reads the ACI statement. "We remain committed to keeping kids safe with a strong reminder that all household cleaning products should be kept out of reach of children."

In 2013 the ACI launched a campaign to educate consumers on how to safely use and store laundry detergent pods. According to the ACI statement, in the first quarter of 2015, incidences of exposure to unit-dose laundry detergents declined in the United States and Europe.

Eco-friendly oil herders

A new type of surfactant derived from a plant-based molecule could help corral oil spills on the ocean surface, according to a recent paper in the journal *Science Advances* (http://dx.doi. org/10.1126/sciadv.1400265, 2015). Unlike other so-called "chemical herders," the surfactant is biodegradable, and its main component is naturally abundant in the marine environment.

Chemical herders are amphiphilic molecules that, when sprayed onto water surrounding an oil spill, cause the oil slick to retract into a thick mass that can be burned away. The herders work by forming a monolayer on the water's surface. When the

herder reaches the edge of the an oil slick, it lowers the air-water surface tension, causing the oil slick to retract and form a thicker layer over a smaller area.

Existing herders, primarily silicone polyethers, are nonbiodegradable and can persist in the marine ecosystem for years, possibly harming aquatic life. So researchers led by George John at the City University of New York (USA) devised a biodegradable herder based on a natural plant molecule. Phytol, a component of chlorophyll, is a regularly branched chain of isoprene units that terminates in a hydroxyl group. To this hydrophobic chain the team added a hydrophilic, cationic group (either *N*-methyl imidazole or pyridine).

The researchers tested the two phytol-based herders in fresh and salt water at three temperatures: cold (5°C), ambient (20°C), and warm (35°C). Although both herders were effective, the one containing the N-imidazole group performed the best, increasing the thickness of an oil slick by about 1,000%, 1,000%, and 2,500% at 5°C, 20°C, and 35°C, respectively, in both salt and fresh water. This performance is similar to that of the best silicone-based herders.

The herders have a chemical structure that makes them susceptible to hydrolysis between the phytol tail and the cationic head group. Marine plants already release large amounts of phytol into ocean water, and the cationic group is quickly diluted in the water column. Mass spectrometry revealed that the herders degraded completely after one month. "We suggest exploiting the applicability of [the phytol-based] green herders as safe and effective oil spill mitigation chemicals for a sustainable future," say the researchers.

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CEFIC examines interaction of REACH and workplace law

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

Geraint Roberts

Proposals on how EU worker protection legislation and REACH processes, such as authorization and restriction, can interact with each other are being prepared by the European Chemical Industry Council (CEFIC).

The issue has risen up the agenda as more substances have been added to the REACH candidate list of substances of very high concern (SHVCs) and so begun the journey to being made subject to the authorization process. Although REACH says uses of substances, subject to authorization, may be made exempt if the risk is controlled by EU legislation "imposing minimum requirements" (Article 58(2)), in practice, this has never happened.

Earlier this year, a group of European bodies, mainly representing the metals, engineering and automotive sectors, called for the "swift setting" of occupational exposure limits (OELs) for substances, where the risks are limited to

workers rather than affecting consumers or the environment, and, for such substances, to be exempted from the candidate list, authorization, or restriction.

CEFIC, however, was not among the trade bodies which made the call because it thinks there might be a better way forward.

"The conceptual idea behind [the group's proposals] is attractive and could handle many cases, but, within CEFIC, we see that since we've had the first substances proposed for the candidate list in 2008, there have been trials to get exemptions from authorization by referring to the worker protection legislation—and so far this has been successful in zero cases, with no support from member states—and that's why we believe presenting it this way isn't the best strategy," CEFIC REACH director, Erwin Annys, told *Chemical Watch*.

A key issue for some member states is that OELs are too blunt an instrument. "Setting these, instead of making substances subject to authorization or restriction, would not be enough for us," said Frauke Averbeck of the German Federal Institute for Occupational Safety and Health (BAUA). "In most cases we have been looking at, we felt just having an OEL was insufficient because it's just substance not

Tips from inform connect

Tips from inform/connect is a regular Inform column that features tips and other discussion highlights from the community forum board at http://www.informconnect.org/home.

A professional who works at a rendering factory that makes about 90–100 metric tons (MT) of tallow per day observed that the tallow sometimes contained a higher than usual polyethylene (PE) content: 200-300 parts per million (ppm) instead of the usual 50 ppm. This is a concern because PE in tallow is one of the main reasons process problems occur. Community members offered the following simple and low-cost removal tips.

- PE is generally removed with a bleaching filter, filtering with diatomaceous earth, or through precoat filtration (with the help of filter aids, such as cellulose, wood, or perlite).
- Because PE is in solution at normal processing temperatures (80–90°C), controlling the temper-

- ature during filtration is critical. At temperatures lower than 70°C, PE becomes solid and can be removed.
- The lower the temperature during filtration, the more PE will be removed. Typical filtration temperatures are in the range of 50-65°C (depending on the required PE level for the filtered tallow).
- Temperature has an impact on viscosity, requiring key metrics such as the required filter area, filter aid dosing, and filter cycle times to be adjusted.
- A filter with 40–50 m² (or two filters of 30 m² operating in parallel mode) is enough to process 100 MT of tallow per day. This is equal to flow rates of 0.1 MT/m²/h.

use specific, and you look at the authorization process by use—each company has to show for its own use that they are handling the substance safely, and not just obeying a general threshold value or exposure limit."

So, instead of joining the other trade bodies in calling for OELs as an alternative to candidate list allocation, CEFIC has come up with another plan, said Annys—looking at the potential interaction between REACH and worker protection legislation, and the decision-making processes involved.

"If you look at the number of substances for which there is an OEL—and which, in the vast majority of cases, is only an indicative one—and compare it with the number of SVHCs we have already and which will follow in future, you'll see there's a discrepancy," he said.

CEFIC is trying to develop an approach which could apply to all chemicals, only used in industrial environments in well-controlled situations. This, said Annys, is much broader than only considering substances covered by worker protection legislation, where, for example, there is no coverage of PBTs and vPvBs.

"We need to work on a much more holistic approach, looking at the potential interactions between REACH and worker protection legislation. It is very clear we have to improve them, including some of the decision-making processes and the choice of substances that are picked up for these laws."

CEFIC hopes to publish its proposals in the autumn, possibly as early as September.

Meanwhile, a call for the European Commission to present a proposal for a revision of the EU Directive on protecting workers from exposure to carcinogens and mutagens is included in a draft European Parliament Resolution on the EU strategic framework on health and safety at work for 2014–20.

The draft, which is being discussed by the Parliament Employment Committee, says the proposal should add more binding OELs and also asks the Commission to develop a system to assess their values, based on "clear and explicit criteria."

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Sunflower wax recovery and edible films applications

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

Leslie Kleiner

Sunflower (*Helianthus annuus* L.) is an important oilseed crop around the world. As a sunflower oil crushing and producing country, Argentina is not only interested in its oil but also in the byproducts that are produced from the recovery of processing waste. The PLAPIQUI (Chemical Engineering Pilot Plant) division of the National Research Council in Argentina (CONICET) performs research and development to advance the recovery of such oilseed processing waste and byproducts as waxes, lecithins, and bioactive components, and also to further applications for these recovered ingredients in the food industry.

To learn more about CONICET-PLAPIQUI's research in sunflower byproduct recovery, I interviewed Erica Baümler, and assistant professor at Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina. Baümler specializes in the recovery of sunflower wax [1] and its applications in edible films [2,3]. Edible films are mostly used in food preservation to control moisture, gases, lipid migration, and other parameters that may affect shelf life [4]. The use of recovered sunflower waxes in edible films is an appealing strategy to incorporate recovered ingredi-

ents from oilseed processing and implement them in main stream applications.

Q: What are sunflower waxes?

Sunflower waxes are natural components found in sunflower seeds that result from the esterification of long-chain fatty acids with long-chain alcohols (36–50 carbons in alcohol chain). These waxes are extracted along with sunflower oil during the oil extraction

process, and they represent minor components in the oil. The content of waxes in the oil depends on the type of seed hybrid, the hull, and the method of oil extraction (pressed or solvent extraction). In crude sunflower oil, the wax content may vary between 50 to over 1,500 ppm, depending on the origin and hybrid of the seed, as well as on the dehulling efficiency and crushing methods.

Q: How are sunflower waxes obtained?

Currently, the objective of sunflower crushing is primarily focused on oil recovery. Therefore, the waxes can be obtained from recovering the waste that arises from sunflower oil purification. In Argentina, for example, waxes are eliminated in the degumming process and are not suitable for sale. Winterization is the common process for the removal of waxes from the oil. These waxes can then be recovered from the purification waste. In this process, agitation is applied at low temperatures (~ 4 °C) to produce a cake of crystallized product. However, the gums can coat the waxes and lower the yield of recovery in the cake. Alternatives to this process include more efficient methods for hull removal or a hexane wash of the seed prior to crushing. We worked on solvent washing methods, which not only would allow thee sunflower waxes to be recovered more efficiently, but would also lead to a lower wax content in the hull during crushing. This technique can lead to the combined effects of wax recovery and lower wax content oil after crushing.

How can sunflower waxes be used in edible films?

In our research, we developed edible films based on the use of calcium pectinate and sunflower waxes. We wanted to retain the properties of the pectin, while imparting a moisture barrier arising from the waxes. Although the moisture barrier presented some cracks that were observed by SEM (scanning electron microscopy), the films were flexible and suitable for handling and packaging. Due to its promising results, this is a continued area of interest in our research.

What are other uses of sunflower waxes?

A:

Sunflower waxes can be used in various applications such as oleogels and edible films. At PLAPIQUI, one of our interests is to study oil refin-

Further reading

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ing by-products for their use in improved and novel applications. Also at PLAPIQUI, we study the processing of fruits and vegetables as well as the enzymatic modification of oils and their derivatives. Having this platform allows us to focus on our specific area of interest and collaborate with our colleagues to research various applications. In our research, we continue to study these sunflower waxes in edible films of diverse formulations, with the objective of developing edible films of various properties.

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.



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Lipid class compositions of plant tissues

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

The compositions of the lipids of plant tissues have been reviewed [1]. Some results are listed in Table 1. Those plant tissues that serve as major food materials have received most study obviously. Triacylglycerols tend to be the most abundant class of storage lipid in tissues that are rich in lipids, such as the commercially important oil seeds. On the other hand, there are exceptions and jojoba oil, for example, consists mainly of wax esters. Many storage tissues in plants have starch as

the main constituent rather than lipid, and in potato tubers and apples the complex glycolipids and phospholipids are the only lipids present at low levels. In addition to those lipids listed, sterols, sterol esters, acylated sterol glycosides, phytoglycolipid, ceramide, glucosylceramide, phosphatidic acid, *N*-acylphosphatidylethanolamine, and phosphatidylserine, amongst others, may be found. It should be recognized that seeds and tubers do not have a homogeneous lipid distribu-

TABLE 1. The lipid class compositions (weight % of the total lipids) of various plant tissues.

Lipid class	Potato tuber	Apple fruit	Soybean seed	Clover leaves	Rye grass	Spinach cloroplasts
monogalactosyldiacylglycerol	6	1	trace	46	39	36
digalactosyldiacylglycerol	16	5	trace	28	29	20
sulfoquinovosyldiacylglycerol	1	1	trace	4	4	5
triacylglycerol	15	5	88			
phosphatidylcholine	26	23	4	7	10	7
phosphatidylethanolamine	13	11	2	5	5	3
phosphatidylinositol	6	6	2	1	2	2
phosphatidylglycerol	1	1	trace	6	7	7
others	15	42	5	3	4	
Reference	[2]	[3]	[4]	[5]	[1]	[6]

tion, the endosperm, germ, bran, and other organelles each having a distinctive composition. The glycosyldiacylglycerols, such as mono- and digalactosyldiacylglycerols and sulfoquinovosyldiacylglycerol, are the most abundant lipid classes in leaf (photosynthetic) tissues. Glycerophospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylglycerol, are also present and other complex lipids are occasionally reported. Phosphatidylglycerol appears to be especially characteristic of photosynthetic tissue, and it can be the main glycerophospholipid in certain green algae. Triacylglycerols are virtually absent from leaves.

As in animal tissues, each of the membranes or organelles in the leaf has a characteristic lipid composition. Spinach chloroplasts have received a great deal of attention, because they can be prepared relatively easily for biochemical experiments, and like the intact leaf they contain appreciable amounts of the glycolipids and a smaller proportion of glycerophospholipids. In contrast, as in animal tissues, the plasma membrane has a high content of phosphatidylcholine, while the mitochondria contain cardiolipin.

A further region of plants with a distinctive composition is the epidermis or cuticle. The lipids here tend to be rich in waxes, and can include cutin and suberin, which are complex polyesters of hydroxy fatty acids.

Further reading

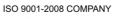
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Leaf surface lipids are largely non-polar waxes. It has been argued that these may be the most abundant lipids on Earth, considering that leaves cover much of the land surface.

This Lipid Snippet document is based on part of Chapter 1 of the Third edition of Lipid Analysis by the author and published by P.J. Barnes & Associates (The Oily Press Ltd). It was omitted from the Fourth edition of the book to save space.



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PATENTS

Sealing material and method for preparing the same

Yamamoto T., Bridgestone Corp., US8906274, December 9, 2014

A sealing material is provided, which has excellent sealing property, with cracks, fracture, and deformation being minimized. The sealing material of the invention is prepared by vulcanizing and foaming a mixture containing a rubber component containing a copolymer rubber of ethylene, an α -olefin having 3 or more carbon atoms, and a non-conjugated diene, a vulcanizing agent, and a blowing agent, and then subjecting the mixture to a cell breakage treatment, characterized in that the mixture further includes a fatty acid calcium salt and a fatty acid zinc salt.

Frozen aerated confections

Greenacre, J.H., et al., Conopco, Inc. and Unilever, US8906443, December 9, 2014

A frozen aerated confection having an overrun of at least 40 percent and a fat component in an amount of 2 to 20% (by weight of the frozen aerated confection), said fat component comprising triglycerides of fatty acids wherein no more than 55 percent (by weight of the fatty acids) of the fatty acids in the triglycerides are saturated, less than 8% (by weight of the triglycerides) of the triglycerides are long-chain SSS triglycerides; characterized in that the ratio of the percentage of fat that is solid at 5 °C to the percentage of the fatty acids in the triglycerides that are saturated (by weight of the fatty acids) is greater than 1 and in that the fat component comprises at most 60% bwt cocoa butter or shea nut oil.

Methods of making compositions comprising lecithin oils and NSAIDs for protecting the gastrointestinal tract and providing enhanced therapeutic activity

Lichtenberger, L.M., University of Texas System, US8911752, December 16, 2014

A novel pharmaceutical composition is provided by which nonsteroidal anti-inflammatory drugs (NSAIDs) are added directly to phospholipid-containing oil such as lecithin oils or to a bio-compatible oil to which an phospholipid has been added to make a NSAID-containing formulation that possess low gastrointestinal (GI) toxicity and enhanced therapeutic activity to treat or prevent inflammation, pain, fever, platelet aggregation, tissue ulcerations, and/or other tissue disorders. The composition of the invention are in the form of a non-aqueous solution, paste, suspension, dispersion, colloidal suspension, or in the

form of an aqueous emulsion or microemulstion for internal, oral, direct, or topical administration.

Biogenic fuel and method of making same

Rusek, J.J., Swift Development Renewable Fuels LLC, Swift Enterprises, Ltd., and Swift Fuels LLC, US8907150, December 9, 2014

A method of producing from a biomass mesitylene-isopentane fuel is provided. A biomass may be fermented to form acetone. The acetone is converted in a catalytic reactor to mesitylene and mesityl oxide. The mesitylene is separated in a phase separator and the organic face containing mesityl oxide is sent to a dehydration reactor, then to a demethylation reactor, and finally to a hydrogenation reactor from which isopentane is recovered. This isopentane is then mixed with the mesitylene to form the final mesitylene-isopentane fuel. The catalytic reaction with acetone employs catalysts of either niobium, vanadium or tantalum.

Enzymes useful for peracid production

Payne, M.S. and R. Dicosimo, E.I. Du Pont De Nemours and Co., US8911977, December 16, 2014

Acetyl xylan esterases and variants thereof having perhydrolytic activity are provided for producing peroxycarboxylic acids from carboxylic acid esters and a source of peroxygen. Multi-component peracid generation systems comprising an enzyme catalyst having perhydrolytic activity are also provided, as are methods of using the present enzyme catalyst to produce peroxycarboxylic acids. The polypeptide having perhydrolytic activity may be used to produce peroxycarboxylic acids suitable for use in a variety of applications such as cleaning, disinfecting, sanitizing, bleaching, wood pulp processing, paper pulp processing, and personal care applications.

Enhanced biodiesel process

Roa-Espinosa, A. and H. Lin, Soilnet LLC, US8907113, December 9, 2014

A process for separating glycerin from methyl ester at an enhanced rate is disclosed. The improved process results from carrying out the transesterification reaction in a substantially non-polar and water free environment. A polymer selected from a group of polymers shown to be effective in such an environment is added to the product mixture which greatly improves the rate of separation between the methyl ester and the glycerin and reduces the number of required steps to accomplish the separation.

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

EXTRACTS & DISTILLATES

Effect of rosemary (Rosmarinus officinalis) extracts on the oxidative stability and sensory acceptability of soybean oil

Dias, L.S., et al., J. Sci. Food Agric. 95: 2021–2027, 2015, http://dx.doi.org/10.1002/jsfa.6914.

Plant extracts have been used as an alternative to the use of synthetic antioxidants in order to preserve oils from oxidative degradation. Additionally, these extracts add special flavors and aromas to the food. Thus, the objective of this study was to evaluate the effect of hydroethanolic extracts of fresh and freeze-dried rosemary in the oxidative stability of soybean oil under accelerated storage in an oven. The application of the extracts in the oil showed that that freeze-dried extract was better in reducing the formation of oxidation products, showing 8.6 meq kg⁻¹ of peroxides after 20 days of storage. On the other hand, the mixture of the natural extract with *t*-butylhydroquinone conferred better oxidative stability index until the 20th day, 9.7 h. Both extracts prevented the loss of tocopherol, not differing between each other (P > 0.05), and present approximately 505 mg kg⁻¹ of residual tocopherols. The sensory evaluation revealed that consumers accepted equally the oils added and not added of the rosemary extracts. The extracts are therefore potential sources of natural antioxidants and they would be well accepted by consumers if applied by the food industry to replace synthetic antioxidants.

Modulation of sphingolipid metabolism with calorie restriction enhances insulin action in skeletal muscle

Obanda, D.N., et al., J. Nutr. Biochem. 26:687–695, 2015, http://dx.doi.org/10.1016/j.jnutbio.2015.01.007.

This study sought to investigate the effect of calorie restriction (CR) on skeletal muscle sphingolipid metabolism and its contribution to improved insulin action in rats after a 6-month feeding study. Twenty nine (29) male Fischer 344 rats were randomized to an *ad libitum* (AL) diet or 30% CR. Dietary intake, body weight and insulin sensitivity were monitored. After 6 months, skeletal muscle (*vastus lateralis*) was obtained for insulin signaling and lipid profiling. CR significantly decreased insulin and glucose levels and also altered the expression and activity of proteins involved in sphingolipid formation and metabolism. The quantities of ceramides significantly increased in CR

animals (P<.05; n=14–15), while ceramide metabolism products (i.e., glycosphingolipids: hexosylceramides and lactosylceramides) significantly decreased (P<.05; n=14–15). Ceramide phosphates, sphingomyelins, sphingosine and sphingosine phosphate were not significantly different between AL and CR groups (P=ns; n=14–15). Lactosylceramide quantities correlated significantly with surrogate markers of insulin resistance (homeostasis model of assessment on insulin resistance) (r=0.7; P<.005). Products of ceramide metabolism (glycosphingolipids), known to interfere with insulin signaling at elevated levels, were significantly reduced in the skeletal muscle of CR animals. The increase in insulin sensitivity is associated with glycosphingolipid levels.

δ-Tocotrienol treatment is more effective against hypoxic tumor cells than normoxic cells: potential implications for cancer therapy

Shibata, A., et al., J. Nutr. Biochem. 26: 832–840, 2015, http://dx.doi.org/10.1016/j.jnutbio.2015.02.011.

Tocotrienols, unsaturated forms of vitamin E, inhibit the proliferation of a variety of cancer cells and suppress angiogenesis. However, the mechanisms underlying those effects on cancer cell growth remain unclear especially under hypoxic conditions. In this study, we demonstrated that δ -tocotrienol (δ -T3) could be used as a novel anticancer agent against human colorectal adenocarcinoma (DLD-1) cells under both normoxic and hypoxic conditions. δ -T3 inhibited the growth of DLD-1 cells in a dosedependent fashion by inducing cell cycle arrest and apoptosis. This effect was more potent under hypoxic than normoxic conditions. The anticancer effect of δ -T3 was achieved by its up-regulation of cyclin-dependent kinase inhibitors (p21 and p27), the activation of caspases and the suppression of phosphorylation of protein kinase B (Akt) at Thr³⁰⁸ and Ser⁴⁷³. In in vivo studies, oral administration of rice bran tocotrienol (RBT3, mainly γ -T3) (10 mg/ mouse/day) significantly inhibited tumor growth in nude mice. In tumor analyses, RBT3 activated p21, p27, caspase-3 and caspase-9 and decreased Akt phosphorylation. Furthermore, immunostaining revealed that RBT3 decreased the number of cells positive for CD31/platelet endothelial cell adhesion molecule-1 in microvessels in the tumor. Taken together, these data suggest that tocotrienols are potent antitumor agents capable of inducing apoptosis and inhibiting angiogenesis under both hypoxic and normoxic conditions. Tocotrienols could have significant therapeutic potential in the clinical treatment of tumors.

Plasma carotenoids and risk of breast cancer over 20 years of follow-up

Eliassen, A.H., et al., Am. J. Clin. Nutr. 101: 1197–1205, 2015, http://dx.doi.org/10.3945/ajcn.114.105080.

Increasing evidence suggests that carotenoids, which are micronutrients in fruit and vegetables, reduce breast cancer risk.

CONTINUED ON NEXT PAGE

Whether carotenoids are important early or late in carcinogenesis is unclear, and limited analyses have been conducted by breast tumor subtypes. We sought to examine issues of the timing of carotenoid exposure as well as associations by breast tumor subtypes. We conducted a nested case-control study of plasma carotenoids measured by using reverse-phase high-performance liquid chromatography and breast cancer risk in the Nurses' Health Study. In 1989-1990, 32,826 women donated blood samples; in 2000-2002, 18,743 of these women contributed a second blood sample. Between the first blood collection and June 2010, 2188 breast cancer cases were diagnosed (579 cases were diagnosed after the second collection) and matched with control subjects. RRs and 95% CIs were calculated by using conditional logistic regression adjusted for several breast cancer risk factors. Higher concentrations of α -carotene, β -carotene, lycopene, and total carotenoids were associated with 18-28% statistically significantly lower risks of breast cancer (e.g., β-carotene top compared with bottom quintile RR: 0.72; 95% CI: 0.59, 0.88; *P*-trend < 0.001). Associations were apparent for total carotenoids measured ≥10 y before diagnosis (top compared with bottom quintile RR: 0.69; 95% CI: 0.50, 0.95; P-trend = 0.01) as well as those <10 y before diagnosis (RR: 0.79; 95% CI: 0.64, 0.98; P-trend = 0.04, *P*-interaction = 0.11). Carotenoid concentrations were strongly inversely associated with breast cancer recurrence and death (e.g., β-carotene top compared with bottom quintile RR: 0.32; 95% CI: 0.21, 0.51; P-trend < 0.001) compared with not recurrent and not lethal disease (*P*-heterogeneity < 0.001). In this large prospective analysis with 20 y of follow-up, women with high plasma carotenoids were at reduced breast cancer risk particularly for more aggressive and ultimately fatal disease.

Field production, purification and analysis of high-oleic acetyl-triacylglycerols from transgenic Camelina sativa

Liu, J., et al., Ind. Crops Prod. 65: 259-268, 2015, http://dx.doi.org/10.1016/j.indcrop.2014.11.019.

A diacylglycerol acetyltransferase, EaDAcT, from Euonymus alatus, synthesizes sn-3 acetyl triacylglycerols (acetyl-TAG) when expressed in Arabidopsis, Camelina and soybean. compared to most vegetable oils, acetyl-TAGs have reduced viscosity and improved cold temperature properties that confer advantages in applications as biodegradable lubricants, food emulsifiers, plasticizers, and 'drop-in' fuels for some diesel engines. A high-oleic Camelina line was engineered to express the EaDAcT gene in order to produce acetyl-TAG oils with fatty acid compositions and physiochemical properties complementary to wild-type acetyl-TAG. The accumulation of acetyl-TAGs at 70 mol% of seed TAG in field-grown high-oleic Camelina had minor or no effect on seed weight, oil content, harvest index and seed yield. The total moles of TAG increased up to 27% reflecting the ability to synthesize more acetyl-TAG from the same supply of long-chain fatty acid. Acetyl-TAG could be separated from long-chain TAG by silica column or by reverse phase chromatography. The predominant acetyl-TAG molecular species produced in high-oleic Camelina was acetyl-dioleoyl-glycerol. The crystallization temperature of higholeic acetyl-TAG (by differential scanning calorimetry at 1.0 °C/

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Journal of Surfactants and Detergents (July)

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min) was reduced by 30 °C compared to control TAG. The viscosity of high-oleic acetyl-TAG was 27% lower than TAG from the high-oleic control and the caloric content was reduced by 5%. Field production of T4 and T5 transgenic plants yielded over 250 kg seeds for oil extraction and analysis.

Triacylglycerol analysis in human milk and other mammalian species: small-scale sample preparation, characterization, and statistical classification using HPLC-ELSD profiles

Ten-Doménech, et al., J. Agric. Food Chem. 63: 5761–5770, 2015, http://dx.doi.org/10.1021/acs.jafc.5b01158.

In this work, a method for the separation of triacylglycerols (TAGs) present in human milk and from other mammalian species by reversed-phase high-performance liquid chromatography using a core–shell particle packed column with UV and evaporative light-scattering detectors is described. Under optimal conditions, a mobile phase containing acetonitrile/*n*-pentanol at 10 °C gave an excellent resolution among more than 50 TAG peaks. A small-scale method for fat extraction in these milks (particularly of interest for human milk samples) using minimal amounts of sample and reagents was also developed. The proposed extraction protocol and the traditional method were compared, giving similar results, with respect to the total fat and relative TAG contents. Finally, a statistical study based on linear discriminant analysis on the TAG composition of different types of milks (human, cow, sheep, and goat) was carried out to differentiate the samples according to their mammalian origin.

Comprehensive and quantitative profiling of lipid species in human milk, cow milk, and a phospholipid-enriched milk formula by GC and MS/MS^{ALL}

Sokol, E., et al., Eur. J. Lipid Sci. Technol. 117: 751–759, 2015, http://dx.doi.org/10.1002/ejlt.201400575.

Here we present a workflow for in-depth analysis of milk lipids that combines gas chromatography (GC) for fatty acid (FA) profiling and a shotgun lipidomics routine termed MS/MS^{ALL} for structural characterization of molecular lipid species. To evaluate the performance of the workflow we performed a comparative lipid analysis of human milk, cow milk, and Lacprodan PL-20, a phospholipidenriched milk protein concentrate for infant formula. The GC analysis showed that human milk and Lacprodan have a similar FA profile with higher levels of unsaturated FAs as compared to cow milk. In-depth lipidomic analysis by MS/MS^{ALL} revealed that each type of milk sample comprised distinct composition of molecular lipid species. Lipid class composition showed that the human and cow milk contain a higher proportion of triacylglycerols (TAGs) as compared to Lacprodan. Notably, the MS/MS^{ALL} analysis demonstrated that the similar FA

profile of human milk and Lacprodan determined by GC analysis is attributed to the composition of individual TAG species in human milk and glycerophospholipid species in Lacprodan. Moreover, the analysis of TAG molecules in Lacprodan and cow milk showed a high proportion of short-chain FAs that could not be monitored by GC analysis. The results presented here show that complementary GC and MS/MS^{ALL} analysis is a powerful approach for characterization of molecular lipid species in milk and milk products.

Beyond diazomethane: alternative approaches to analyzing non-esterified fatty acids

Potter, G., et al., Eur. J. Lipid Sci. Technol. 117: 908–917, 2015, http://dx.doi.org/10.1002/ejlt.201400404.

In many branches of lipid science, researchers are interested in non-esterified fatty acid (NEFA) content and composition. For many years, diazomethane was the reagent of choice to selectively derivatize and then detect NEFA due to its highly specific methylation of the carboxylic acid functional group. While the activity of this derivatizing reagent is very defined, it is dangerous and can be difficult to obtain. In this brief review, we have compiled a collection of methods which allow for the detection of NEFA and hydroxy NEFA without the use of diazomethane. We have concentrated on methods that employ three distinct approaches of selective quantification/extraction, purification from total lipids and derivatization techniques. Chemical reactions that occur during selective extraction of NEFA using a quaternary ammonium salt (a) and the later pyrolytic derivatization when the mixture is placed in the hot injection port of a GC (b).

Potential role of milk fat globule membrane in modulating plasma lipoproteins, gene expression, and cholesterol metabolism in humans: a randomized study

Rosqvist, F., et al., Am. J.Clin. Nutr. 102: 20–30, 2015, http://ajcn.nutrition.org/content/102/1/20.full.

Butter is rich in saturated fat [saturated fatty acids (SFAs)] and can increase plasma low density lipoprotein (LDL) cholesterol, which is a major risk factor for cardiovascular disease. However, compared with other dairy foods, butter is low in milk fat globule membrane (MFGM) content, which encloses the fat. We hypothesized that different dairy foods may have distinct effects on plasma lipids because of a varying content of MFGM. We aimed to investigate whether the effects of milk fat on plasma lipids and cardiometabolic risk markers are modulated by the MFGM content. The study was an 8-wk, single-blind, randomized, controlled isocaloric trial with 2 parallel groups including overweight men and women (n = 57 randomly assigned). For the intervention, subjects consumed 40 g milk fat/d as either whipping cream (MFGM diet) or butter oil (control diet). Intervention foods were matched for total fat, protein, carbohydrates, and calcium. Subjects were discouraged from consuming any other dairy products during the study. Plasma markers of cholesterol absorption and hepatic cholesterol metabolism were assessed together with global gene-expression

analyses in peripheral blood mononuclear cells. As expected, the control diet increased plasma lipids, whereas the MFGM diet did not [total cholesterol (\pm SD): +0.30 \pm 0.49 compared with -0.04 \pm 0.49 mmol/L, respectively (P = 0.024); LDL cholesterol: $+0.36 \pm 0.50$ compared with $+0.04 \pm 0.36$ mmol/L, respectively (P = 0.024); apolipoprotein B:apolipoprotein A-I ratio: $+0.03 \pm 0.09$ compared with -0.05 \pm 0.10 mmol/L, respectively (P = 0.007); and non-HDL cholesterol: $+0.24 \pm 0.49$ compared with -0.14 ± 0.51 mmol/L, respectively (P =0.013)]. HDL-cholesterol, triglyceride, sitosterol, lathosterol, campesterol, and proprotein convertase subtilisin/kexin type 9 plasma concentrations and fatty acid compositions did not differ between groups. Nineteen genes were differentially regulated between groups, and these genes were mostly correlated with lipid changes. In contrast to milk fat without MFGM, milk fat enclosed by MFGM does not impair the lipoprotein profile. The mechanism is not clear although suppressed gene expression by MFGM correlated inversely with plasma lipids. The food matrix should be considered when evaluating cardiovascular aspects of different dairy foods. This trial was registered at clinicaltrials.gov as NCT01767077.

LIPID OXIDATION

Ultrasound-assisted extraction of oil from rice bran: a response surface methodology approach

Krishnan, V.C.A., et al., J. Food Process. Technol. 6: 454-461, 2015, http://dx.doi.org/10.4172/2157-7110.1000454.

In the present study, ultrasound-assisted extraction of oil from rice bran was investigated through response surface methodology (RSM), where process variables were amplitude level (50-100%) and sonication time (5-30 min). It was found that all process variables had significant (p<.05) effects on the response variable. A central composite design (CCD) was used to determine the optimum process conditions. Optimal conditions were identified as 93% amplitude level and 26 min sonication time for maximum crude oil yield of 10.8%. It was found that oil yield by ultrasound-assisted extraction using ethanol as solvent was higher than that of the conventional method using hexane or ethanol as solvent. Furthermore peroxide value and free fatty acid values were comparable in all the extracted samples. GC-MS analysis confirmed that ultrasound treated samples had a higher percentage of unsaturated fatty acids compared to conventional extraction using hexane. It was also observed that solvent extraction using ethanol showed higher percentage of the unsaturated fatty acid compared to hexane as solvent. This study confirms that ultrasound assisted extraction using ethanol as solvent can retain maximum unsaturated fatty acid compared to conventional hexane extracted sample, which is a food grade solvent. Hence in conclusion ultrasound assisted extraction has the potential to replace the conventional solvent extraction

Controlling the stability of chocolates through the incorporation of soft and hard StOSt-rich fats

Tran, P.D., et al., Eur. J. Lipid Sci. Technol., available online only, accepted July 3, 2015, http://dx.doi.org/10.1002/ejlt.201400584.

The presented study investigates the functionality of hard and soft StOSt-rich fats in plain and hazelnut-based filled dark chocolates. Blends of cocoa butter (CB) with different StOSt-rich fats, namely Vietnamese mango fat (VMF), Indian mango fat (IMF), its stearin (IMFst) and olein fraction (IMFol) were selected for application in these chocolate products based on their phase and crystallisation behaviour. It was shown that a fat phase formulation with CB/VMF 70/30 and CB/ IMFst 70/30 increased the heat resistance of dark chocolate and maintaining similar chocolate quality attributes (colour, hardness, melting and flow properties) compared to the CB reference. Furthermore, these fat blends increased the fat bloom stability following oil migration, as shown by visual assessment by a trained panel, cryo-SEM imaging and oil migration monitoring by HPLC-ELSD. In addition, the fat blend CB/IMFol 90/10, suitable for chocolate applications under nontropical conditions, showed to retard oil migration fat bloom as well. Distinct mechanisms for the observed phenomena were proposed. Furthermore, the different steps of fat bloom development, starting from the appearance of oil blisters to the presence of crystals (\sim 30 μ m) on the chocolate surface were captured using cryo-SEM.

Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications

Luo, X., et al., Mar. Drugs 13: 4231-4254; 2015, http://dx.doi.org/10.3390/md13074231.

Microalgae contain a variety of bioactive lipids with potential applications in aquaculture feed, biofuel, food and pharmaceutical industries. While microalgae-derived polyunsaturated fatty acid (PUFA) and their roles in promoting human health have been extensively studied, other lipid types from this resource, such as phytosterols, have been poorly explored. Phytosterols have been used as additives in many food products such as spread, dairy products and salad dressing. This review focuses on the recent advances in microalgae-derived phytosterols with functional bioactivities and their potential applications in functional food and pharmaceutical industries. It highlights the importance of microalgae-derived lipids other than PUFA for the development of an advanced microalgae industry.

Fundamentals of electrospinning as a novel delivery vehicle for bioactive compounds in food nanotechnology

Ghorani, B. and N. Tucker, Food Hydrocolloids 51: 227-240, 2015, http://dx.doi.org/10.1016/j.foodhyd.2015.05.024.

Encapsulation of bioactive compounds and probiotic bacteria within prebiotic substances to protect or even enhance their survival whilst passing upper gastro-intestinal tract, is an area of great interest for both academia and the food industries. Different methods have been suggested, examined and applied to encapsulate and dry probiotics and bioactive compounds, for example spray drying. However, the harsh processing conditions of these methods can significantly reduce the viability of bacteria or damage the structure of the target molecules. Electrospinning (and the related process of electrospraying) both show promise as a novel delivery vehicle for supplementary food compounds

because the process can work with an aqueous solution, at room temperature and without coagulation chemistry to produce matrices in the micro- and nano-range. The production of nanofibers (fiber diameters less than 1 µm) is a commonplace. Nanofiber materials produced by electrospinning have attracted particular attention in the food industry because of their potential as vehicles for sustained and controlled release. The room temperature process route is compatible with food grade polymers and biopolymers, and allows efficient encapsulation by reducing denaturation, and enhancing stability of bioactives. Consequently, there is clear potential to develop electrospun fibrous assemblies to advance the design and performance of novel products and delivery systems for supplementary food compounds. To optimize production conditions and maximize throughput, a clear understanding the mechanism of electrospinning is essential. This paper presents a comprehensive review of the fundamentals of electrospinning to produce nanofibers suitable for food technology application particularly for use in encapsulation and as nano-carriers.

The role of oxygen in lipid oxidation reactions: a review

Johnson, D.R. and E.A. Decker, *Annu. Rev. Food Sci. Technol.* 6: 171–190, 2015, http://dx.doi.org/10.1146/annurev.

The susceptibility of food oil to quality loss is largely determined by the presence of oxygen. This article reviews the current understanding concerning the effect of oxygen types, location, and concentration on the oxidative stability of foods. It also discusses the major factors that influence the interaction between oxygen and lipids such as antioxidants, prooxidants, reactive oxygen species (ROS), environmental conditions, and oxygen scavengers. Research has shown that the amount of oxygen needed to cause oxidation is generally very small and that by reducing oxygen concentration in containers to less than 2%, oxidative stability can be greatly enhanced. However, very few studies have systematically examined the oxygen levels needed to reduce, or inhibit, lipid oxidation process. Thus, a more comprehensive understanding of the relationship between oxygen levels and lipid oxidation is necessary for the development of innovative antioxidant solutions and package designs that prolong the quality of foods containing lipids.

Micronutrients in vegetable oils: the impact of crushing and refining processes on vitamins and antioxidants in sunflower, rapeseed, and soybean oils

Fine, F., et al., Eur. J. Lipid Sci. Techn., online first July 23, 2015, http://doi.org/10.1002/ejlt.201400400.

Tocopherols, phytosterols, polyphenols, and coenzymes Q are naturally present in oilseeds such as sunflower, rape and soybean. Besides contributing to taste and color, micronutrients help protect against health disorders such as cardiovascular diseases and cancer. However, during the conventional oil manufacturing process, many minor components are lost. Given that diet is a major cause of cardiovascular diseases and cancer, it makes sense to optimize the content of micronutrients in food, and specifically in vegetable oils. These micronutrients have antioxidant properties that inhibit the oxidation of

low-density lipoprotein cholesterol. This review summarizes important recent research emphasizing the impact of crushing and refining processes on the micronutrient content of different vegetable oils. After the crushing step, the total sterol content was high in crude rape, sunflower and soybean oils, at $4358-10\ 569$, 2212-4146 and 1735-4328 mg/kg, respectively. The tocopherol content was lower, at 464-1458, 725-1892 and 1094-2484 mg/kg, respectively, and the level of phenolics was 113-629, 10-120, and 23-148 mg/kg, respectively. The refining process destroyed micronutrients: 10-36% loss of total tocopherols, 6-52% loss of total sterols and 93-98% loss of polyphenols. Studies have focused on improving the extraction of tocopherols by alternative heating (microwave treatment, roasting or steaming processes). These treatments improved tocopherol content, and extraction was faster and consumed less energy.

Frying stability of sunflower oil blended with jujube (*Ziziphus mauritiana* Lam.) leaf extract

Delfanian, M., et al., Food Sci. Nutr., online first May 19, 2015, http://doi.org/10.1002/fsn3.247.

The aim of present study was to compare the effects of ultrasoundassisted and microwave- assisted extraction with solvent extraction method on antioxidant activities of jujube (Ziziphus mauritiana Lam.) leaf extracts in stability of sunflower oil during deep frying. The antioxidant activities of the extracts were evaluated by using 2, 2- diphenyl- 1- picrylhydrazyl (DPPH ') radical scavenging and β-carotene bleaching assays. Ultrasound- assisted extraction was the most effective method on antioxidant activities of extracts and extraction yield of phenolic compounds compared to other extraction techniques. Protective effect of methanol-water extract of jujube leaf obtained with ultrasound- assisted extraction (ULMW) at 500 and 700 ppm in stability of sunfl ower oil was compared to synthetic antioxidants by measuring total polar compounds (TPC), carbonyl value (CV), peroxide value (PV), free fatty acids (FFA), oxidative stability index (OSI), conjugated dienes (CD), and trienes values (CT). Results showed ULMW at 700 ppm had higher stabilization effi ciency than synthetic antioxidants.

Development of iron-chelating poly(ethylene terephthalate) packaging for inhibiting lipid oxidation in oil-in-water emulsions

Johnson, D. R., et al., J. Agric. Food Chem. 63: 5055–60, http://doi.org/10.1021/acs.jafc.5b00796.

Foods such as bulk oils, salad dressings, and nutritionally fortified beverages that are susceptible to oxidative degradation are often packaged in poly(ethylene terephthalate) (PET) bottles with metal chelators added to the food to maintain product quality. In the present work, a metal-chelating active packaging material is designed and characterized, in which poly(hydroxamic acid) (PHA) metal-chelating moieties were grafted from the surface of PET. Biomimetic PHA groups were grafted in a two-step UV-initiated process without the use of a

photoinitiator. Surface characterization of the films by attenuated total reflective Fourier transform infrared spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM) suggested successful grafting and conversion of poly(hydroxyethyl acrylate) (PHEA) to PHA chelating moieties from the surface of PET. Colorimetric (ferrozine) and inductively coupled plasma mass spectroscopy (ICP-MS) assays demonstrated the ability of PET-g-PHA to chelate iron in a low-pH (3.0) environment containing a competitive metal chelator (citric acid). Lipid oxidation studies demonstrated the antioxidant activity of PETg-PHA films in inhibiting iron-promoted oxidation in an acidified oilin-water (O/W) emulsion model system (pH 3.0). Particle size and ζ-potential analysis indicated that the addition of PET-g-PHA films did not affect the physical stability of the emulsion system. This work suggests that biomimetic chelating moieties can be grafted from PET and effectively inhibit iron-promoted degradation reactions, enabling removal of metal-chelating additives from product formulations.

Anti- and pro-oxidative effect of fresh and freeze-dried vegetables during storage of mayonnaise

Raikos, V., *et al.*, *J. Food Sci. Technol.*, online only, accepted June 3, 2015, http://doi.org/10.1007/s13197-015-1897-x.

Abstract Mayonnaise was supplemented with vegetables (5 % w/w) and the effect of storage time at 4 °C on the oxidative stability of the dispersed phase was investigated. Results indicated that mayonnaise is prone to lipid oxidation during storage under refrigerator conditions. The type of vegetable used for mayonnaise reformulation was critical in inhibiting oxidation and followed the order beetroot > carrot \approx onion with respect to antioxidant capacity. Broccoli induced a prooxidant effect and the rate of oxidation by the end of the storage period was 42 times higher compared with the control. The addition of beetroot, either fresh or freeze-dried, improved the oxidative stability of mayonnaise significantly. The process of freeze-drying affected adversely the ability of vegetables to decrease oil oxidation of the emulsions. This may reflect loss of important natural antioxidants during the drying procedure.

Rapid screening of mixed edible oils and gutter oils by matrix-assisted laser desorption/ ionization mass spectrometry

Ng, T.T., et al., Anal. Chim. Acta 884: 70–76, 2015, http://dx.doi.org/10.1016/j.aca.2015.05.013.

Authentication of edible oils is a long-term issue in food safety, and becomes particularly important with the emergence and wide spread of gutter oils in recent years. Due to the very high analytical demand and diversity of gutter oils, a high throughput analytical method and a versatile strategy for authentication of mixed edible oils and gutter oils are highly desirable. In this study, an improved matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) method has been developed for direct analysis of edible oils. This method involved on-target sample loading, automatic data acquisition and simple data processing. MALDI-MS spectra with high quality and high reproducibility have been obtained using this method, and

a preliminary spectral database of edible oils has been set up. The authenticity of an edible oil sample can be determined by comparing its MALDI-MS spectrum and principal component analysis (PCA) results with those of its labeled oil in the database. This method is simple and the whole process only takes several minutes for analysis of one oil sample. We demonstrated that the method was sensitive to change in oil compositions and can be used for measuring compositions of mixed oils. The capability of the method for determining mislabeling enables it for rapid screening of gutter oils since fraudulent mislabeling is a common feature of gutter oils.

INDUSTRIAL APPLICATIONS

Aqueous enzyme-assisted oil extraction from oilseeds and emulsion de-emulsifying methods: a review

Yusoff, M.M., et al., Trends Food Sci. Technol. 41: 60–82, 2015, http://doi.org/10.1016/j.tifs.2014.09.003.

Regulatory, safety, and environmental issues have prompted the development of aqueous enzymatic extraction (AEE) for extracting components from oil-bearing materials. The emulsion resulting from AEE requires de-emulsification to separate the oil; when enzymes are used for this purpose, the method is known as aqueous enzymatic emulsion de-emulsification (AEED). In general, enzyme assisted oil extraction is known to yield oil having highly favourable characteristics. This review covers technological aspects of enzyme assisted oil extraction, and explores the quality characteristics of the oils obtained, focussing particularly on recent efforts undertaken to improve process economics by recovering and re-using enzymes.

Comparison of ethyl acetate with hexane for oil extraction from various oilseeds

Lohani, M.C., et al., Journal of the American Oil Chemists' Society 92: 743–754, 2015,

http://doi.org/10.1007/s11746-015-2644-1.

The aim of the current research was to determine a less hazardous, cheaper and less toxic alternative solvent for hexane for extraction of oil from different oilseeds showing equivalent oil yield and oil quality. A full factorial design with three levels of extraction temperature (80, 100 and 120 °C) and three levels of extraction time (40, 65 and 90 min) with constant solvent to seed ratio value of 4:1 was used to extract the oil. Maximum oil was recovered from canola followed by flax, mustard and camelina. The oil content of canola was found in range of 21.08–36.44, and 25.12–40.38 % for hexane and ethyl acetate, respectively. The heating values of oil extracted from all oilseeds using hexane and ethyl acetate were found in the range of 38.04–39.98 and 37.98–39.37 MJ/kg, respectively. Least viscosity was found for flax seed using hexane followed by camelina, canola and mustard as compared to ethyl acetate. Viscosity of flax oil ranged from 27.23–37.19, and 31.16–55.52 cP for hexane and ethyl acetate solvents, respectively.

Considering human safety, less environmental impact, comparable oil yield and quality parameters, ethyl acetate can be a promising alternative to hexane.

Extraction of coriander oil using twin-screw extrusion: feasibility study and potential press cake applications

Uitterhaegen, E., et al., Journal of the American Oil Chemists' Society, published online, June 30, 2015, http://doi.org/10.1007/s11746-015-2678-4.

This study presents an assessment of the vegetable oil extraction from coriander fruits through mechanical pressing, more specifically twin-screw extrusion. This comprises an evaluation of the oil recovery obtained and its respective quality, as well as the specific mechanical energy, representing an economical point of view. With regard to the extrusion optimization, the screw configuration, the device's filling coefficient and the pressing temperature were varied. The screw configuration was shown to exhibit a key influence on the extraction efficiency and oil recoveries of at least 40 % were reached when the pressing zone was positioned immediately after the filter and consisted of 50 mm long, reverse screws with a -33 mm pitch. Furthermore, with a device's filling coefficient of 39.4 g/h rpm and a pressing temperature of 120 °C, an oil recovery of 47 %, the highest of this study, was reached with concurrent low energy consumption. Next to this, operating parameters of 47.1 g/h rpm and 80 °C resulted in the production of a press cake with the lowest residual oil content (15 %) in this study, although this also involved a significant increase in the filtrate's foot content. All the produced oils were of acceptable quality (<1.5 % acidity), showed high petroselinic acid content (73 %), and were pleasantly scented.

Fate of contaminants during the refining process of vegetable oils and fats. a calculation model

van Duijn, G. and Vlaardingen, Eur. J. Lipid Sci. Technol., online first July 7, 2015,

http://doi.org/10.1002/ejlt.201500170.

Crude vegetable oils and fats after oil extraction may contain contaminants introduced in the supply chain. Many of these contaminants are largely removed from the oil by the refining process. However, these contaminants will concentrate in the refinery by-products; acid oil from soapsplitting and filter blowing, spent bleaching earth, and deodorizer distillate. The contaminant levels in these by-products are calculated by determining the lipids balance of the refinery and the fate of the contaminants in the refining process. Calculations of some example oils with characteristic contaminant levels show that deodorizer distillate from chemical refining has a high contamination risk, deodorizer distillate from physical refining and spent bleaching earth has a medium risk, while acid oil from soapsplitting and filter blowing have a low contamination risk.

SYNTHETIC BIOLOGY

Metabolic engineering of oilseed crops to produce high levels of novel acetyl glyceride oils with reduced viscosity, freezing point, and calorific value

Liu, J., et al., Plant Biotechnol. J. 13: 858–865, 2015, http://doi.org/10.1111/pbi.12325.

Seed oils have proved recalcitrant to modification for the production of industrially useful lipids. Here, we demonstrate the successful metabolic engineering and subsequent field production of an oilseed crop with the highest accumulation of unusual oil achieved so far in transgenic plants. Previously, expression of the Euonymus alatus diacylglycerol acetyltransferase (EaDAcT) gene in wild-type Arabidopsis seeds resulted in the accumulation of 45 mol% of unusual 3-acetyl-1,2-diacylsn-glycerols (acetyl-TAGs) in the seed oil (Durrett et al., 2010 PNAS 107:9464). Expression of EaDAcT in dgat1 mutants compromised in their ability to synthesize regular triacylglycerols increased acetyl-TAGs to 65 mol%. Camelina and soybean transformed with the EaDAcT gene accumulate acetyl-triacylglycerols (acetyl-TAGs) at up to 70 mol% of seed oil. A similar strategy of coexpression of EaDAcT together with RNAi suppression of DGAT1 increased acetyl-TAG levels to up to 85 mol% in field-grown transgenic Camelina. Additionally, total moles of triacylglycerol (TAG) per seed increased 20%. Analysis of the acetyl-TAG fraction revealed a twofold reduction in very long chain fatty acids (VLCFA), consistent with their displacement from the sn-3 position by acetate. Seed germination remained high, and seedlings were able to metabolize the stored acetyl-TAGs as rapidly as regular triacylglycerols. Viscosity, freezing point and caloric content of the Camelina acetyl-TAG oils were reduced, enabling use of this oil in several nonfood and food applications.

Metabolic pathway engineering towards enhancing microalgal lipid biosynthesis for biofuel application—a review

De Bhowmick, G., et al., Renew. Sust. Energ. Rev. 50: 1239–1253, 2015, http://doi.org/10.1016/j.rser.2015.04.131.

Microalgae have recently emerged as the most favorite feedstock for triacylglycerol (TAG), the storage neutral lipid, for renewable and sustainable production of biodiesel, mainly due to their comparable lipid contents, faster growth rates and lesser land requirements as compared to the non-conventional and non-edible oilseed crops. But the real technological challenge is to mass produce microalgae with much higher lipid content to make the production of a low-value-high-volume product like biodiesel economically viable and environmentally sustainable. Recent scientific achievements in TAG

overproduction in higher eukaryotic systems may be leveraged upon to enhance lipid synthesis by manifold in microalgae. Since the available sequence homology information have been effectively used in case of the model unicellular green alga, *Chlamydomonas reinhardtii* to perform genome-scale metabolic reconstructions, the gained knowledge and the well-established genetic engineering tools and techniques coupled with the modern system biology approaches may well pave the way for delineating and deciphering the TAG biosynthetic pathways in lipid accumulating microalgae as targets for metabolic pathway engineering. This review thus analyzes the trends and developments in the area of metabolic engineering of lipid synthesis in microalgae and discusses the vision based on some of the possible strategies that could be adopted to reconstruct a stable modified engineered microalga with enhanced lipid producing capabilities. The

strategies include flux balance analysis for target gene identification, over expression of the target enzymes involved in lipid biosynthesis, over expression of the target gene under specific inducible promoters, constitutive expression of transcription regulators, diverting the flux of key metabolites, and integrated *in silico* based approaches. An integrated approach involving multiple gene targeting by applying the principles and knowledge of systems biology and bioinformatics would provide us with a holistic view and help derive some feasible solutions.

Bob Moreau (US Department of Agriculture) and Bryan Yeh (Intrexon) are regular contributors to Extracts & Distillates.

Energy (cont. from 508)

innovative system to absorb carbon dioxide from the flue gas of a nearby power plant.

- Arizona State University (Mesa) will receive up to \$1 million for atmospheric carbon dioxide capture, enrichment, and delivery to increase biomass productivity.
- The University of California, San Diego will receive up to \$760,000 to develop an automated early detection system that can identify and characterize infestation or infection of an algae production pond in order to ensure crop health.
- Lawrence Livermore National Laboratory (Livermore, California) will receive up to \$1 million to protect algal crops by developing probiotic bacteria to combat pond infestation and increase ecosystem functioning and resilience.

Renewable energy targets quadrupled globally since 2005

Renewable energy targets are now a defining feature of the global energy landscape, according to a new report by the International Renewable Energy Agency (IRENA; Abu Dhabi, UAE).

Renewable Energy Target Setting finds that 164 countries have embraced at least one type of renewable energy target, up from just 43 countries in 2005. Two more countries, Canada and the United Arab Emirates, have set renewable energy targets at the subnational level.

Developing and emerging economies are leading the adoption of targets, accounting for 131 of the 164 countries with renewable energy objectives. The majority of countries focus on the electricity sector—150 countries have renewable electricity targets—but commitments in other sectors are also on the rise. The number of countries setting targets for the heating/cooling sector increased from two countries in 2005 to 47 today. Similarly, renewable transport targets have more than doubled from 27 countries in 2005 to 59 today.

While underscoring the importance of renewable energy targets, the report recognizes that they are not sufficient in and of themselves. In order to be seen as credible by investors and society and to provide a reliable trajectory for the future evolution of the energy mix, they

need to be accompanied by a clear strategy and backed by specific policies and measures.

Visit http://tinyurl.com/IRENA2015 for the report (PDF).

Volkswagen renewable diesel evaluation program completed

Volkswagen of America Inc. announced the successful completion of its Renewable Diesel Evaluation Program in collaboration with Solazyme Inc. and Amyris Inc., two manufacturers of renewable fuels and products.

Beginning in 2012, Volkswagen measured the environmental impacts from the use of renewable diesel formulas with TDI (Turbocharged Direct Injection) Clean Diesel technology found in the 2012 Passat TDI (which uses a NO $_{\rm x}$ storage system) and 2012 Jetta TDI (selective catalytic reduction system). Initial analysis found that advanced renewable fuels in the test offered comparable performance to standard crude-based diesel fuel blends while producing fewer CO $_{\rm x}$ emissions on average.

During the two-year evaluation, Solazyme's now commercialized Soladiesel RD (100% algae-derived renewable diesel fuel) and the Amyris plant-sugar derived renewable diesel formula were used for the program, with each company testing a 2012 Passat TDI and Jetta TDI models. Both fuel producers used additives to meet ASTM D975 specifications.

With more than 134,000 miles logged collectively in real-world, on-road, and on-highway conditions, Volkswagen engineers found that every vehicle in the evaluation offered similar performance to existing TDI powertrains operating on today's crude-based clean diesel fuels. While powered by the two fuel formulas, results from the evaluation found that driving dynamics were not negatively impacted while fuel economy was similar or improved. In addition to comparable performance, both producers claim that greenhouse gas emissions could be reduced by more than 50% on a well-to-wheel basis when using renewable fuels as compared to today's commercially available crude-based fuels.

Color measurement made easy

Björn Hotting

In the detergent industry, the color changes standardized stains undergo when they are washed are a strong parameter that can be used to improve products and create new formulas. Traditionally, most labs use spectrophotometers to measure color. Although these instruments are accurate, they are not easy to use and must be operated by skilled technicians. In the detergent industry, a small wash test typically involves 20 different stains with two repetitions and two wash

- Color is a strong parameter that can be used to improve products and create new formulas.
- Although the spectrophotometers currently used to measure color in the detergent industry are accurate, they are also slow, require a lot of repetition, and can only be operated by skilled technicians.
- This article describes a new camerabased color measurement technology that not only measures color accurately, but also quickly, with less repetition, and with easy-to-use software that can be operated by unskilled workers.

loads. Consequently, a small wash test of four different detergent formulas requires 320 color measurements—possibly even 640, if color is measured both before and after washing. This is a simple but time-consuming job which, due to the accuracy required and the complexity of the equipment, cannot be done by unskilled workers.

The Mach5 is a camera-based instrument that can measure the color of several areas simultaneously (Fig. 1). These measurement areas can be rectangular, round, or any other shape. The user sees a live image of the object and can use the mouse to position the measurement areas. The instrument measures absolute color and gives output in L^* , a^* , b^* and X, Y, Z values (see sidebar on page 542).

In the wash test mentioned above, the Mach5 can measure 20 samples in one pass. This means that only 16 cycles of measurement (instead of 320) are needed. Fewer measurements speed up the overall process and make it more user-friendly. Data can easily be exported, and users can even automate their (statistical) calculations (by adding their own Python script, for instance) to combine, move, or prepare data for custom reporting. (Python is an easy to learn programming language that allows users to write complete software or use inside existing software, as one would use a macro within Microsoft Excel. Information is available at https://www.python.org/.)

Measuring 320 color samples one after another with a traditional spectrophotometer is physically and mentally tedious, and it can be difficult to find highly skilled technicians who want to repeat the same small task eight hours a day.

In contrast, the user-friendly interface of the Mach5 software does not require a high level of skill. Automatic warnings prevent errors, and the software helps users position the measurement locations, which can be stored for future use.





DISHWASHER TESTING AND SUB AREAS

The new camera-based system addresses another challenge in color measurement: The stains in dishwashers are not removed evenly during the washing process. The typical dishwasher test material is a small 120mm by 100mm plate. A spectrophotometer with a 9mm or even a 20mm opening will measure only a small area of the plate and average the results. Consequently, the results will vary largely depending on where the measurement is made, and any information about how and where the stain is removed will be lost. While it is possible to measure different locations, adding multiple spectrophotometer measurements to one dishwasher tile only increases the workload.

Instead of providing an average measurement for the entire test material based on one location, the Mach5 automatically splits the measurement area into smaller sub-areas and calculates the standard deviation for the various sub-areas, providing critical information about the homogeneity of the total area.

ACCURACY

Most cameras only divide the spectrum of visible light into 3 parts (red, green, and blue) and are therefore not very good at measuring color in absolute terms. The technology in the Mach5 is upgraded to measure color better by splitting the visible light spectrum into 25 parts instead of only three (Fig. 2, page 542).

CONTINUED ON NEXT PAGE



FIG. 1. Mach5 with open drawer. The object to measure can either be inserted via the drawer or via the two doors. In the image, the standard color checker card is measured. This also gives an idea about the dimensions of the drawer size and measurement area.

A quick introduction to color measurement

There are various ways to measure color. A tri-stimulus instrument divides the fully visible spectrum of light into three parts: usually red, green, and blue. Having only three values allows such instruments to measure quickly. Their chief limitation is that they do not detect subtle color changes within colors, such as different shades of red, blue, or green; only the total sum is given.

The human eye can detect visible light ranging in wavelength from blue (380nm) to red (680nm). A spectrophotometer that divides this range into 10nm intervals will have 30 data points over the full range, which is 10 times the data points the tri-stimulus sensor has. These additional data points allow the spectrophotometer to "see" the more subtle changes within red, green or blue.

The best instrument to use depends on the application. For example, the granulate used to make polyethylene terephthalate (PET) bottles is checked by the suppliers, so it should be spectrally correct. Because PET bottle manufacturers only add more or less of a color additive to the plastic, it is very unlikely that color changes within red, green, or blue will occur. In most cases, only an intensity change will occur as a result of adding too much or too little of the color additive. On the other hand, in the detergent industry, where multiple stains are washed together, real color changes (not just in intensity) are observed. Also, the stains are not standard dyes but real live food ingredients, so a tri- stimulus approach will not yield the best results.

There are various ways to describe color. Most systems give a color value with three numbers. A well-known system is the simple red, green, blue system (RGB) used in PCs and televisions. These are non-absolute systems; for instance, if the backlight of the screen is changed the same RGB value will result in a very different color.

In addition to non-absolute systems there are absolute color terms such as L*,a*, b* and X,Y,Z. These allow a designer in the United States to communicate with a manufacturer in China about color in a common language they both understand.

Absolute color is defined by three coordinates. In the L*,a*b* color space, L* is the lightness (from black to white), a* is the red-green axis, and b* is the blue-yellow axis. The L*,a*b* system is designed in such a way that a 1-point change is perceived equally by humans. So, a 1-point change in a* (red-green shift) will be seen equally as a 1-point change on either the L* or b* axis. For more info on color spaces like XYZ and L*,a* and b* see: http://en.wikipedia.org/wiki/Lab_color space.

The Mach5 is slightly less accurate than a spectro (dE < 2), but the major benefit of the Mach5 over conservative spectrophotometers is its flexibility with respect to the shape and size of the areas that can be measured, as well as the extra information it gives. It can also be operated by an unskilled worker, and is quicker than a spectrophotometer, as it can measure multiple

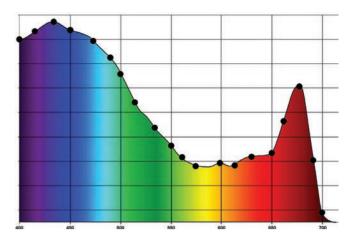


FIG. 2. The spectral data of a sample as measured by the Mach5, which show the visible light spectrum from blue to red. The black dots represent measurement points in the spectrum. Normal camera-based systems only have three data points (red, green and blue). The Mach5 has multiple data points per spectrum, which makes it considerably more accurate.

areas in one cycle. Full-size images of measured objects are saved for later assessment. The software and some of the hardware can be adapted to provide additional functionalities for specific industries.

Although the current version of the Mach5 can only measure the color of non- gloss materials, a new version that handles gloss materials will be released by end of 2015.

READING BARCODES ON CODED TEST MATERIAL

Codes are traditionally hand written on test materials so that samples before and after washing can be uniquely identified and their results compared.

Since the Mach5 uses a camera, it can also read barcodes. Some test material suppliers have already begun to offer a service in which they add a barcode to the test material. The Mach5 can read that barcode at the same time it measures the color, uniquely identifying the product and saving the data by the code that appears on the test material.

CUSTOMERS AND MARKETS

The first Mach5 was sold five years ago. That first customer asked for a market-specific instrument. Colour Consult designed the instrument, wrote the software, and helped the customer sell it.

Because of that, most customers are in the detergent industry, but the company is considering expanding into other markets. The instrument is sold worldwide as a turnkey system with the PC included, so the instrument can easily be serviced remotely. A short video is available on request; please contact Colour Consult for more information.

Bjorn Hotting is the owner of Colour Consult. He can be contacted at BHotting@ColourConsult.nl.

Mastering the art

of the abstract

Crystal Snyder

Imagine you've just arrived at a conference. Ahead of you are three full days of concurrent sessions, a hundred presentations to choose from, your carry-on bag still stuffed with papers you meant to read on the plane, and new publication alerts piling up your inbox while you are gone. Where do you begin? Or perhaps, you're starting to wonder, when will it ever end?

It's a familiar dilemma. We may thrive on new knowledge, but we're also drowning in it. There never seems to be enough time to read or attend everything of value. Sometimes, the best we can hope for is to tread water by reading abstracts.

Yet, the art of writing a great abstract remains an underrated skill. Too often, the abstract is written in haste after we've completed the "real work" of collecting, analyzing, and interpreting our data. We expect our results to speak for themselves, but without an effective abstract, they instead risk being overlooked amid the swelling tide of scholarly communication.

At its best, an abstract does much more than summarize your results. These few words, if well crafted, can create a compelling first impression that forces your audience to take note. A great abstract is, in essence, an invitation.

So, how can you put your abstract to work for you??

An effective abstract has four essential components, which can be easily remembered using the acronym "CARS." Just as cars need all four wheels to keep moving, an effective abstract needs all four of its essential components—context, approach, results, and significance (CARS)—to be persuasive.

"C" FOR CONTEXT: WHAT'S THE PROBLEM?

The beginning of your abstract should provide context by answering the question: "What is this study about and why is it needed right now?" Often, an effective abstract will begin with a general statement that establishes the scope of the problem, followed by a more specific question or hypothesis that describes



the focus of the current paper. The goal is to give the reader a sense of the "big picture" and where your research question fits.

What to avoid:

- over-generalizing or stating the obvious, which do little to orient readers to the problem you're aiming to address. Frame your research question in concrete terms, asking yourself, "What does my audience need to know to understand why this research question is important?"
- a mini-literature review. Avoid citing literature in your abstract, unless your research question specifically concerns a previously published paper (you are aiming to reproduce previously reported results, for example).

CONTINUED ON NEXT PAGE

- An effective abstract is more than a summary of results.
- Effective abstracts have four essential components, conveying the context, approach, results, and significance of your research.
- Together, your title and abstract should be able to stand alone and persuade an audience to engage with your work.

For more information

Lebrun, Jean-Luc, *Scientific Writing: A Reader and Writer's Guide.* World Scientific Publishing, Hackensack, New Jersey, USA (2007).

talking "around" your research question. If you start reading
abstracts carefully, you'll be amazed by how often a research
question or hypothesis is implied, but not clearly stated.
The goal of your abstract is to be absolutely clear about your
purpose. State your research question explicitly.

"A" FOR APPROACH: HOW ARE YOU ADDRESSING THE PROBLEM?

In most cases, the next component of the abstract provides an overview of your methods. From the reader's point of view, it should answer, "Are the experimental approaches used here appropriate for the research question?" This portion often provides the reader with an early clue about the validity of your results, as well as a basis for comparison with other studies addressing similar problems.

What to avoid:

 too much detail. Your abstract should convey the broad strokes of your methods, just enough to give your reader a sense of how you approached your research question. Extraneous details are irrelevant to the reader at this stage, eat away at your limited word count, and disrupt the cohesiveness of your abstract.

"R" FOR RESULTS: WHAT DID YOU LEARN?

This is the heart of your abstract, and the first chance for your results to shine. Lead with your strongest, most compelling results. Think about the result you'd be most excited to tell a colleague about in the taxi on the way to the conference. Or imagine your reader taking one thing out of your abstract to share with his or her colleagues. What is that one thing you most want them to remember?

What to avoid:

- vague descriptions or "anticipated" results. This is a common weakness in conference abstracts, as authors hedge their preliminary results against what they hope will be more conclusive results in time for the conference. As scientists, we are necessarily conservative creatures, but as critical consumers of scientific information, we are also moved by what's actually known, not what might be known six months after you've submitted your abstract. Even if some of your work will be completed after the abstract deadline, there needs to be a convincing "hook" in your abstract that will encourage your audience to take a chance on your presentation.
- numbers. Your abstract is about quickly conveying key takehome messages. These are easily lost if readers need to wade through and interpret a lot of numbers without any accompanying visuals. Let your tables, graphs, and other visual images do that work, and make sure that your abstract clearly and concisely summarizes your key conclusions.

"S" FOR SIGNIFICANCE: SO WHAT?

Unfortunately, this is the section most abstracts miss entirely. By this point, your reader understands your problem, your approach, and your key results; now what? Who cares? What can they do with this new information? Why is it relevant? How does this study move the field forward?

This may seem self-evident to us as writers and researchers, but as those closest to our own subjects, we may be tempted to take this familiarity for granted. For newcomers, an abstract that doesn't convey the significance of our work is like a story that ends mid-sentence. By omission, we can easily undo the hard work we've already put into writing an otherwise powerful abstract.

YOUR TITLE AND ABSTRACT SHOULD WORK TOGETHER

Together, your title and abstract are the first thing your audience will see, and frequently, it's all they look at before making a decision about whether to attend a presentation or access the full text of an article. When writing your title and abstract, it's important to consider not just how well it summarizes your work, but also, how effectively it can stand alone, apart from the full text or presentation. Your title should be descriptive of your abstract without being too repetitive. The title should complement and add meaning to the abstract, and *vice versa*.

The title and abstract also play an important role in helping readers discover your work. Although full-text search capabilities are now quite advanced, many databases still index only certain portions, such as the title, abstract, and keywords. Choosing your keywords carefully, and distributing synonymous keywords between your title and abstract can help you avoid direct repetition while increasing the chances that someone will search for one of the keywords you've used.

What to avoid:

- a title that is too long. Readers typically skim titles for useful information, and often judge very quickly whether or not to invest further time. The longer the title, the greater our tendency to skip over it.
- language that is likely to lose its relevance. How many "new," "novel," or "improved" methods exist in the literature that are no longer state-of-the-art? Titles are an enduring identifier for your work. Instead of focusing on novelty, consider using keywords that will retain their longevity.
- catchy titles that lack keywords. While a unique, attention-grabbing title can be extremely effective in prompting readers to keep reading, be careful that you don't sacrifice searchability by leaving out important keywords. Remember, a reader can't love your abstract if they can't find it!

BECOME A CRITICAL READER

If there's a silver lining in the never-ending deluge of new scientific information, it's that you spend plenty of time in the reader's shoes. You are already a discerning reader, but are you aware of what makes you choose one presentation or paper over another? What tips an abstract in your favor? What questions linger in your mind with ineffective or incomplete abstracts? Start applying what you learn to your own work, and you'll soon be on your way to mastering the art of the abstract.

Crystal Snyder is the coordinator of the University of Alberta's Undergraduate Research Initiative and a former laboratory manager in Agricultural Lipid Biotechnology. She can be reached at crystal.snyder@ualberta.ca.



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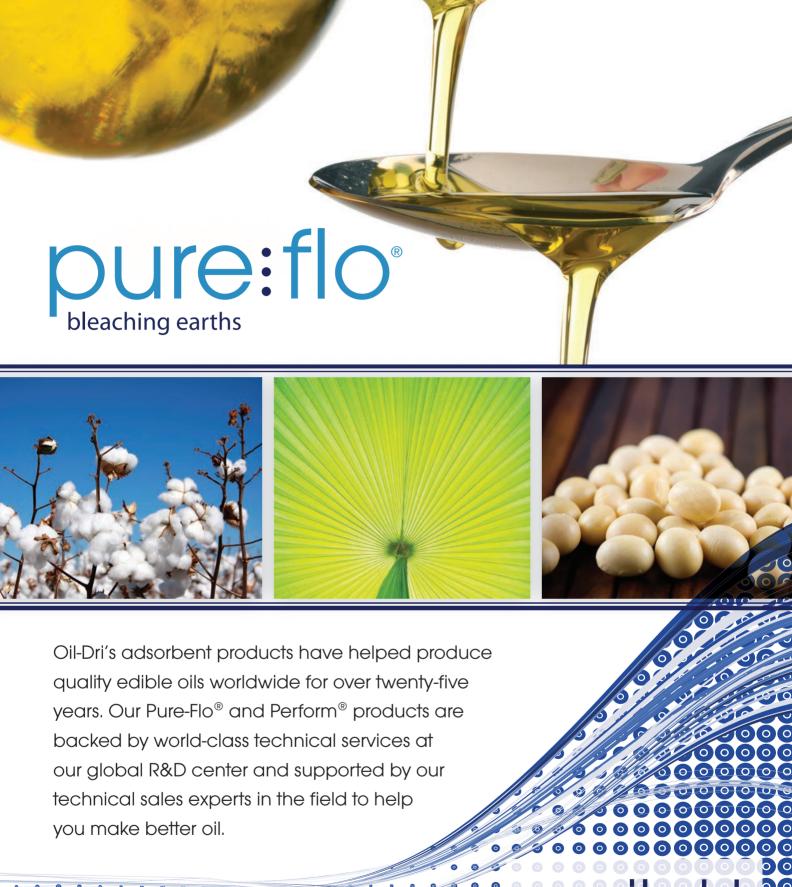
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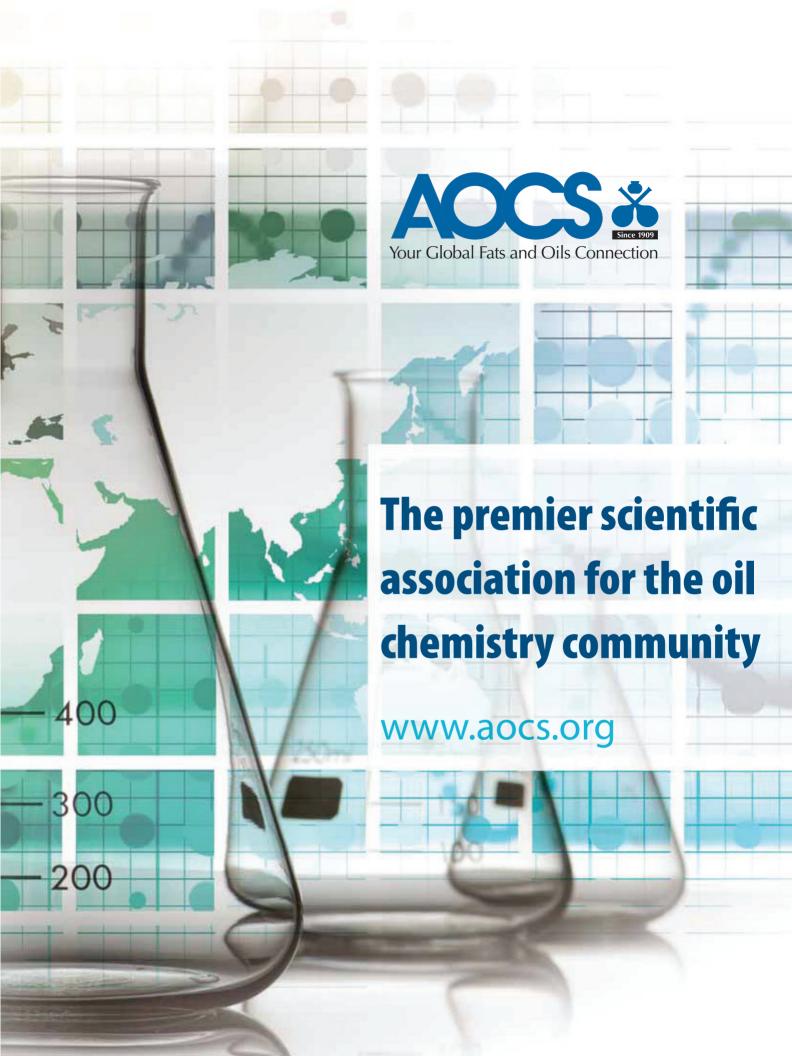
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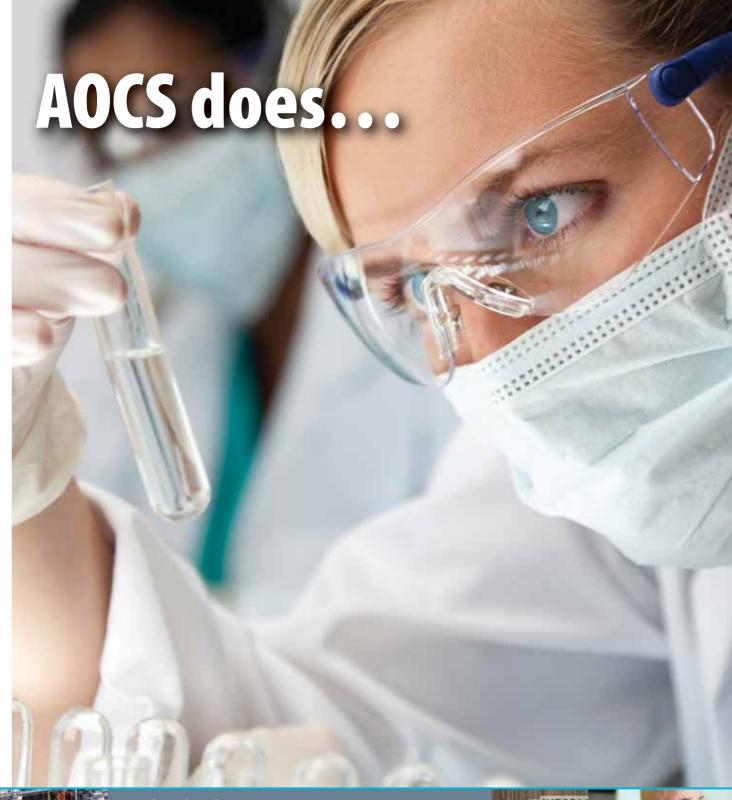
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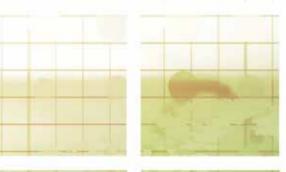
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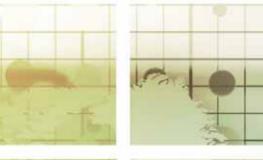
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