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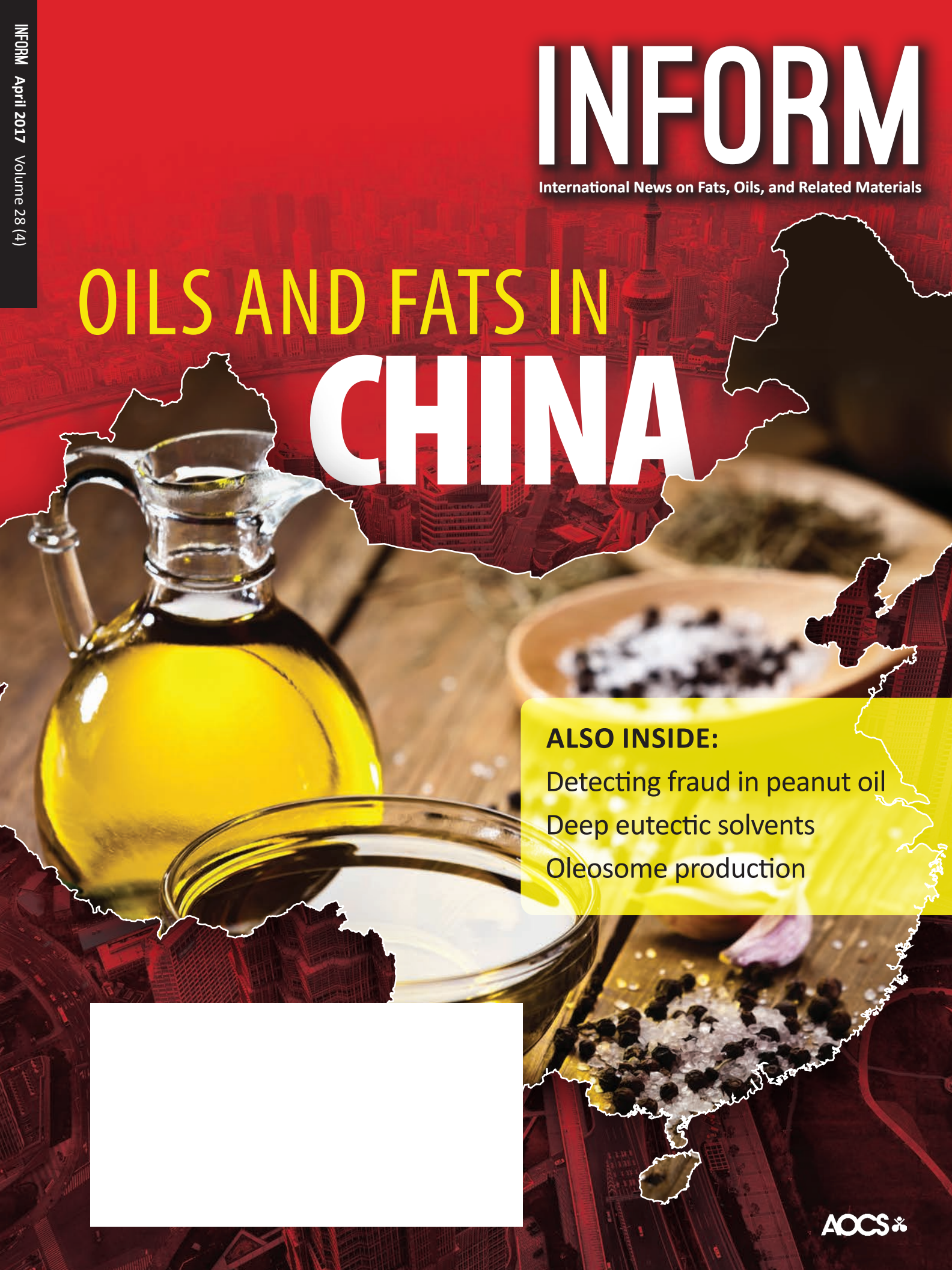

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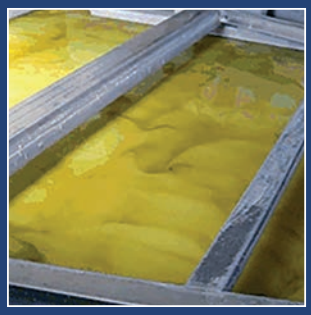

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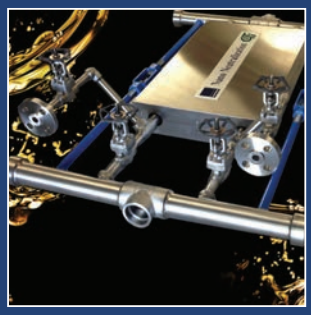

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China's evolving edible oils industry

Laura Cassiday

When Keshun Liu was a child growing up in China, he remembers that his mother used pork lard and locally crushed, unrefined rapeseed oil for cooking. "Lard and locally produced rapeseed oil were used interchangeably in family cooking," says Liu, now a research chemist at the US Department of Agriculture–Agricultural Research Services (USDA–ARS) in Aberdeen, Idaho. "Most families would buy pork, render the fat portion into lard, and use the lard for cooking purposes." Especially in rural areas, if vegetable oil was available at all, it was typically unrefined and unpackaged. However, this situation has changed drastically in a relatively short period of time. "For years now, people do not eat lard anymore," says Liu. "They eat refined and packaged vegetable oil, purchased from the grocery store, which is considered a healthier oil."

- **Patterns of edible oil consumption in China have changed with increased urbanization, improved standards of living, and food safety concerns.**
- **China is now the world's largest consumer, the second-largest producer, and the second-largest importer of edible oils.**
- **Soybean, rapeseed, palm, and peanut oils are the most commonly consumed edible oils in China, although "Western" oils and fats such as olive oil and butter are gaining in popularity.**

Many factors have contributed to this shift in cooking oil preferences, including increased urbanization, improved standards of living, and concerns about food safety. And while much of China is becoming increasingly westernized, cultural and economic factors make China's patterns of edible oil production and consumption unique.

PATTERNS OF CONSUMPTION

China's growing economy and rising incomes led to a 440% increase in per capita vegetable oil consumption in China between 1979 and 1999 (Fang, C., and Beghin, J. C., http://dx.doi.org/10.1006/jcec.2002.1796, 2002). By 1999, China was the world's largest importer of soybean, rapeseed (canola), and palm oils. In 2002, household survey data from 18 Chinese provinces revealed three major regions of vegetable oil consumption (Fang, C., and Beghin, J. C., http://dx.doi.org/10.1006/jcec.2002.1796). Each region had a "staple" vegetable oil that comprised about 80% of total edible oil consumption, with limited consumption of other oils and fats.

In the Northeast region, which consists of the provinces of Heilongjiang, Jilin, and Liaoning (Fig. 1), the staple oil was soybean oil. Rapeseed oil predominated in the Middle and West regions (Jiangsu, Zhejiang, Anhui, Jiangxi, Hunan, Sichuan, Guizhou, Yunnan, Shaanxi, Gansu, Qinghai, and Xinjiang). In the South region, which includes Guangdong, Fuijang, and Guangxi, peanut oil was the most popular. These regional patterns of vegetable oil consumption may be explained by varied climates, land conditions, oilseed production, cultures, and socioeconomic standards. However, as per capita income increases, the pattern of vegetable oil consumption typically diversifies (Fang, C.,

FIG. 1. The provinces of China. Credit: iStock

and Beghin, J. C., http://dx.doi.org/10.1006/jcec.2002.1796, 2002).

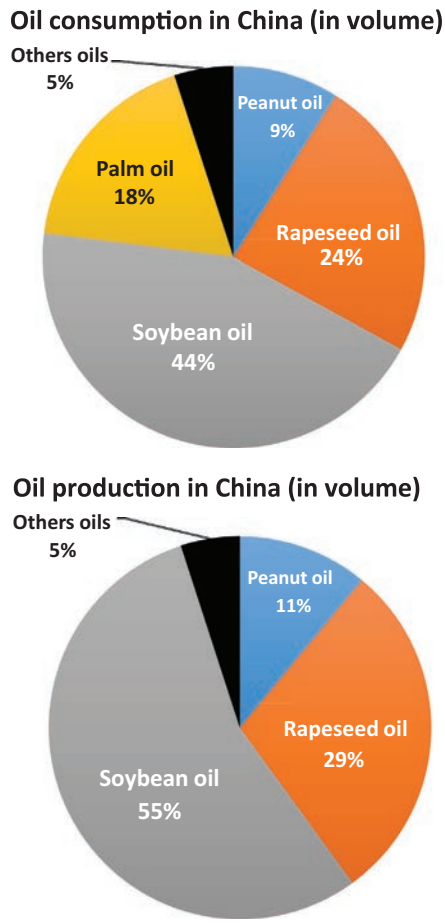
"China is the world's largest consumer of vegetable oils," says Yuanfa Liu, professor of food science at Jiangnan University, in Wuxi, China. "With the constant development of the economy and the increase of total population, the consumption of vegetables oils is increasing year by year." Currently, the top vegetable oils consumed in China as a whole are soybean oil (44%), rapeseed oil (24%), palm oil (18%), and peanut oil (9%), with other oils (cottonseed, sunflower, sesame, camellia, olive, etc.) making up the remaining 5% (Fig. 2, left) (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). Consumers are increasingly accepting "Western" oils and fats, such as olive oil, butter, and margarine. The consumption of palm oil, imported from Malaysia and Indonesia, in China has risen considerably in recent years because it is less expensive than soybean or rapeseed oil and has broad applications in processed foods and fried snacks. Soybean oil, rapeseed oil, and peanut oil are produced domes-

tically in China (Fig. 2, right). As for oilseed production in China, peanut predominates (32%), followed by rapeseed (28%), soybean (23%), sunflower (5%), and other oilseeds (12%).

RISING IMPORTS

In recent years, increasing liberalization of China's economy has affected the edible oil industry. Rationing of vegetable oils in China ended in 1993, and since then the market has been driven by competitive forces (Fang, C., and Beghin, J. C., http://dx.doi.org/10.1006/jcec.2002.1796, 2002). Until 2006, the Chinese government imposed tariff rate quotas (TRQ) on vegetable oil imports to protect domestic products. Quantities of imported edible oils below a specific quota were taxed at one rate (for example, 13%), whereas imports above the quota were subject to a substantially higher tariff (for example, 90%). With accession to the World Trade Organization, China phased out the TRQ for soybean and rapeseed oil, leaving only a 9% import tariff for soybean oil and rapeseed oil imports. TRQ on sunflower, peanut, and corn oil were also eliminated and replaced with a 10% tariff.

FIG. 2. Vegetable oil consumption and production in China. Credit: Jamet, J.-P., and Chaumet, J.-M. (2016) "Soybean in China: adapting to the liberalization." *OCL 23*, D604. http://dx.doi.org/10.1051/ocl/2016044



Source : GEB-Institut de l'Elevage / Clever and Wu, 2016

A growing population combined with free-market forces have brought about dramatic changes in agricultural trade in China. Until the mid-twentieth century, China was self-sufficient in feeding its population (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). However, in the 1960s China began to import cereals, especially wheat, as Chinese agriculture was unable to keep up with demand. Since the 1990s, China has experienced steady economic growth and an increased standard of living, which has caused the economy to move even further away from self-sufficiency.

Until the 1950s, China was the world's largest soybean producer, and the country remained a net soybean exporter until the 1990s (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). In the past, most soy in China was used for human consumption in foods such as tofu, soy sauce, miso, and tempeh. In recent years, increased soybean oil consumption, as well as an increased demand for animal feed (and thus soymeal) to support China's growing livestock industry, have greatly affected the soybean market. With higher incomes and increased westernization, Chinese households are now consuming more meat and dairy products than ever before, increasing the demand for livestock feed and soybean meal. China has been forced to increase soybean imports to keep up with the demand.

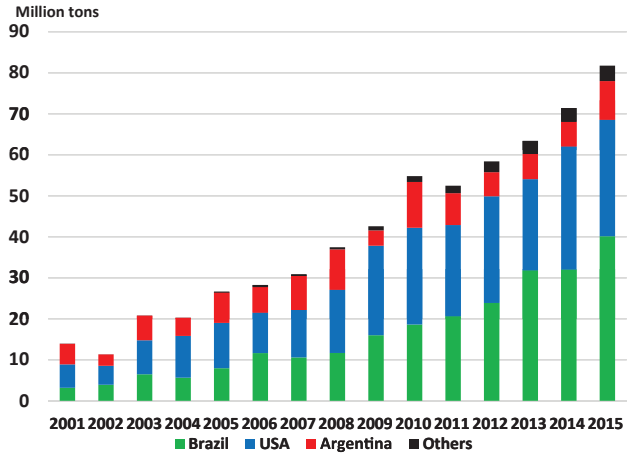
Because arable land in China is limited, the Chinese government made the strategic decision to achieve and maintain independence in cereals at the expense of other crops, such as soybeans (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). In particular, corn production has doubled in the past 15 years, supplanting soybean cultivation in many areas. Whole, unprocessed soybeans are imported for domestic processing. In 1999, the tariff on whole soybean imports was lowered from 180% to 3%. Unlike soybeans, TRQ remain for rice, wheat, and corn, encouraging domestic production. However, even with these challenges, China remains the fourth largest soybean producer in the world.

China is the world's largest importer of soybeans, with 63.5% of the import share in 2013 (Yang, T. K., and Zheng, Y., *Inform 27*, 24–25, 40, 2016). Most soybeans are imported into China from Brazil (49%), the United States, (35%), and Argentina (12%) (Fig. 3). Seventy-five percent of Brazil's, 50% of the United States', and 85% of Argentina's soybean exports are sold to China (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). Most rapeseed oil in China is produced from domestic seed, with rapeseed and rapeseed oil imports coming primarily from Canada.

To assist soybean farmers with rising production costs, in 2008 the Chinese government set a minimum price for domestically produced soybeans (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). As a result, the price of domestic soybeans in China is significantly higher than that of imported beans. In 2013, the price of imported soybeans averaged USD $600 per ton (plus a 3% tariff), compared with a price of USD $750 for domestically produced soybeans. Thus, many Chinese seed crushers have favored imported soybeans over domestic.

To make domestic soybean prices more competitive, in 2014 the Chinese government changed the soybean and rapeseed subsidy system. Now, instead of guaranteeing a minimum price, the system sets a "target price" and provides a "deficiency payment" for the difference between the target price and the market price. Because of the new system, between January 2014 and December 2015 the price of Chinese soybeans fell by 20%, but soybean imports still did not slow due to increased demand for animal feed and oil.

FIG. 3. Chinese soybean imports Credit: Jamet, J.-P., and Chaumet, J.-M. (2016) "Soybean in China: adapting to the liberalization." *OCL 23*, D604. http://dx.doi.org/10.1051/ocl/2016044



Source : GEB-Institut de l'Elevage / TradeMap

China's "Crops structural adjustment plan 2020" aims to reduce the production of corn and encourage farmers to increase planting of peanuts, soybeans, alfalfa, and other crops (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). The plan calls for reducing areas planted with corn by 3.3 million hectares, going back to the 2011 level. In contrast, the soybean surface area will be increased by 2.6 million hectares, restoring the 2006 level. Although these measures will boost domestic soybean production, the need for imported soybeans will likely continue.

CHINA'S SOYBEAN-CRUSHING INDUSTRY

The soybean production areas in China are primarily located in the northeast and central east provinces. Prior to the 1990s, soybean-crushing plants were mostly locally owned and situated in the producing areas. With increasing soybean imports, many new crushing plants were built along the coastal region (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). Because of the lower price of imported soybeans and barriers to transportation for domestic soybeans, it became less expensive for crushing facilities near harbors to purchase imported soybeans.

The large volume of soybean seeds crushed in China (70 million metric tons in 2015) has made the country self-sufficient in soybean meal, and China is a net exporter of soymeal to countries such as Japan, Vietnam, and South Korea (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). However, China remains a net importer of soybean oil. Although China's capacity for edible oil refining has increased dramatically in recent years, and China is the world's largest soybean oil producer, the demand for soybean oil has risen even more rapidly.

Since 2005, the majority of China's soybean-crushing industry has been owned by foreign companies (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). In April 2004, when soybean prices were at a peak, Chinese crushers agreed to buy US soybeans. Six months later,

HOT TOPIC: CHINA'S FAT AND OIL INDUSTRY

Do you want to learn more about edible oils and fats in China? Then make plans to attend the Hot Topic session, "China's fat and oil industry: a fast-growing segment with opportunities and challenges" at the 2017 AOCS Annual Meeting and Industry Showcases April 30–May 3 in Orlando, Florida, USA.

The session, sponsored by the newly established China Section of AOCS, will provide timely updates on the current status of the fats and oils industry in China, including production, consumer trends, research and product development, and markets for edible oils as well as protein co-products. Invited speakers are industry leaders and top scientists in China.

when the payments were due, soybean prices fell dramatically. Many soybean buyers tried to default on their contracts, but an international court ruled against them. The "2004 soybean crisis" resulted in huge monetary losses and the bankruptcy of many Chinese crushing companies. Consequently, many international companies built or purchased crushing facilities in China. Currently, about 60% of China's soybean crushing is performed by international companies such as ADM/Wilmar, Bunge, and Cargill.

CHANGING TIMES

Under China's thirteenth Five-Year Plan (a government blueprint for social and economic policies for the years 2016–2020), China's oil processing technology and manufacturing equipment will expand in scale and become increasingly automated, intelligent, and energy-efficient, says Xuebing Xu from Wilmar Global R&D Center, in Shanghai, China. "China is one of the world's largest edible oil-producing, -consuming, and -importing countries," he says. "China's oil research and oil processing technology, as well as the apparatus, are reaching an internationally advanced level."

In the past, most vegetable oil in China was bought in bulk and suffered from quality and safety issues. "In recent years, the Chinese government has increased its emphasis on food safety," says Ruiyuan Wang from the Chinese Cereals and Oils Association, in Beijing. "Government and industry are working together to reduce unhealthful oil components." In 2011, some large Chinese cities such as Beijing and Shanghai banned the retail sale of bulk edible oils. Higher-quality, refined oils in smaller packages are becoming increasingly common throughout China.

This increased focus on food safety includes enhanced surveillance and penalties for the production and sale of gutter oil—used cooking oil that is illegally collected from restaurant waste and gutters, crudely processed, and sold as a cheaper alternative to new cooking oil. Gutter oil, which has been a major problem in China, Taiwan, Hong Kong, and other countries, often contains toxins and carcinogens that are harmful to human health.

"With the constant development of the economy, increasing income of residents, as well as increased urbanization of China, people are more concerned with the safety and nutri-

Information

Fang, C., and Beghin, J. C. (2002) "Urban demand for edible oils and fats in China: evidence from household survey data." *J. Comp. Econ. 30*, 732–753. http://dx.doi.org/10.1006/jcec.2002.1796

Jamet, J.-P., and Chaumet, J.-M. (2016) "Soybean in China: adapting to the liberalization." *OCL 23*, D604. http://dx.doi.org/10.1051/ocl/2016044

Yang, T. K., and Zheng, Y. (2016) "Chinese edible oils market and consumption trends." *Inform 27*, 24–25, 40. (February 2016)

tion of food and with a healthy diet," says Yuanfa Liu. Edible oils perceived as healthful, including camellia, sunflower, olive, rice bran, and corn oils, are growing in popularity.

However, the Chinese people may not be ready to accept genetically modified (GM) vegetable oils with beneficial fatty acid profiles, such as high-oleic soybean oil. Surveys indicate that the vast majority of the Chinese public is wary of GM crops. The government has officially banned GM commercial crops, and currently only non-GM soybeans are grown in China. More than 60% of these domestic, non-GM soybeans are used in foods such as tofu and soy milk (Jamet, J.-P., and Chaumet, J.-M., http://dx.doi.org/10.1051/ocl/2016044, 2016). Imported GM soybeans are allowed for the production of edible oils and animal feed, but not for food use. Most GM soybeans have a 3–5% higher oil content than non-GM soybeans, as well as lower production costs.

Recently, the Chinese government has indicated an increased willingness to explore GM technology in order to improve domestic crop productivity. "With time and the popularization of GM scientific knowledge, GM vegetable oils rich in beneficial fatty acids will gradually be accepted by consumers," Xu predicts.

Despite the fact that China is becoming increasingly westernized, patterns of edible oil consumption in China still differ substantially from those in North America and Europe. "In the West, we use a lot of butter, margarine, and shortenings in breads and bakery products," says Keshun Liu. "But in China, bakery products are not very common. We normally eat a steamed bread, which does not include shortening as an ingredient." He notes that most modern Chinese kitchens do not contain ovens because baking a cake or bread at home is so uncommon. "The Chinese are going to westernize, but they will still keep their own traditions," says Liu. "Even though some people in the urban population like butter or margarine, and others like French fries, pizza, or fried chicken, the majority of people in the rural areas cannot afford or have access to western food."

Laura Cassiday is an associate editor of Inform *at AOCS. She can be contacted at laura.cassiday@aocs.org.*

A rapid analytical method for detecting fraud in peanut oil

Wang Xin and Zhu Wen-ran

- **Peanut oil is one of the major edible oils in China, but it is prone to adulteration. Consequently, establishing a simple and rapid method to detect adulteration in peanut oil is important.**
- **A new method using low-field nuclear magnetic resonance (LF-NMR) and chemometrics was recently developed to enable the rapid authentication of peanut oil.**
- **The method eliminates the sample pretreatment step, and can reliably detect adulteration of peanut oil with other vegetable oils in a single 5-minute analysis.**

Owing to its pleasant flavor and the presence of compounds such as resveratrol, peanut oil is one of the major edible oils in China, along with soybean oil and rapeseed oil. However, peanut oil is more expensive than the other two oils, making it prone to adulteration by unscrupulous dealers. Such dealers may mix varying proportions of cheaper oils, such as soybean oil, sunflower oil, canola oil, or palm oil into peanut oil or, worse, use cheaper oils to make fake peanut oil by adding peanut oil flavor.

The adulteration of oils not only violates the rights and interests of consumers, food processors, and other industries, but can also lead to potential health risks and the resale of "recycled oil" in China. Consequently, the authentication of vegetable oils and detection of adulteration are important to human health and safety, and many studies have focused on detecting oil adulteration. To the best of our knowledge, techniques available for the rapid authentication and detection of adulteration in peanut oil in particular are still limited. Therefore, establishing a simple and rapid method to detect adulteration in peanut oil is important.

^{1}H Low-field nuclear magnetic resonance (LF-NMR) has been proposed as a rapid, simple, and effective tool for wide use in food quality control and material property measurements. Spin-spin relaxation (T_2) is one of the LF-NMR parameters that represent two features of proton relaxation, and can provide more information about the relaxation time. It has been used to study water mobility in acidified milk drinks, hake muscle after different freezing and storage conditions, and the drying degree and quality of chicken jerky. Moreover, LF-NMR provides a powerful tool to evaluate the quality of deep-frying oil, and there is good correlation between total polar compounds (TPCs), viscosity, and LF-NMR parameters. Nevertheless, there have been very few reports on the measurement of edible oil adulteration by using LF-NMR relaxation measurements.

Our research group recently reported the development of a new method to enable the rapid authentication of peanut oil using low-field nuclear magnetic resonance (LF-NMR) and chemometrics. The method eliminates the sample pretreatment step, and can reliably detect the adulteration of peanut oil with less expensive vegetable oils and fake peanut oils.

An LF-NMR analyzer NMI20-Analyst (Niumag Electric Corporation, Shanghai, China) combined with a Windows analysis platform, and an inversion of a multiexponential fitting analysis (T-invfit) program were used for the NMR measurements (Fig. 1). The strength of the magnetic field was 0.53 T, which corresponded to a proton resonance frequency of 22 MHz.

A comparison of the continuously distributed relaxation spectral curves revealed visible differences between the different oil samples (Fig. 2, page 14). The hydrogen protons in the oil samples could be categorized into two groups according to their relaxation response in the magnetic field, and the two peaks may be attributed to two distinct mobility populations of the protons on the alkyl chain, or inhomogeneous structural organizations with two different packing densities and intermolecular interaction intensities or types. Thus, the difference of the fatty acid composition may have some influence on their T2 spectra. Significant differences in the LF-NMR parameters, single component relaxation time (T_{2W}), and peak area proportion (S_{21} and S_{22}), were detected between pure and adulterated peanut oil samples.

Both the established principal component analysis (PCA) and discriminant analysis (DA) models based on the LF-NMR relaxation results could correctly distinguish authentic peanut

FIG. 1. LF-NMR analyzer (NMI20-Analyst) as described

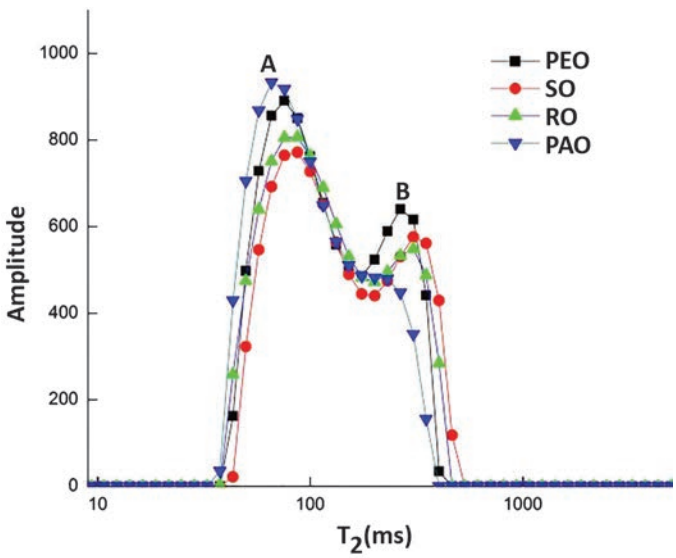


FIG. 2. T2 distributions of four edible oils (PEO: peanut oil; SO: soybean oil; RO: rapeseed oil; PAO: palm oil)

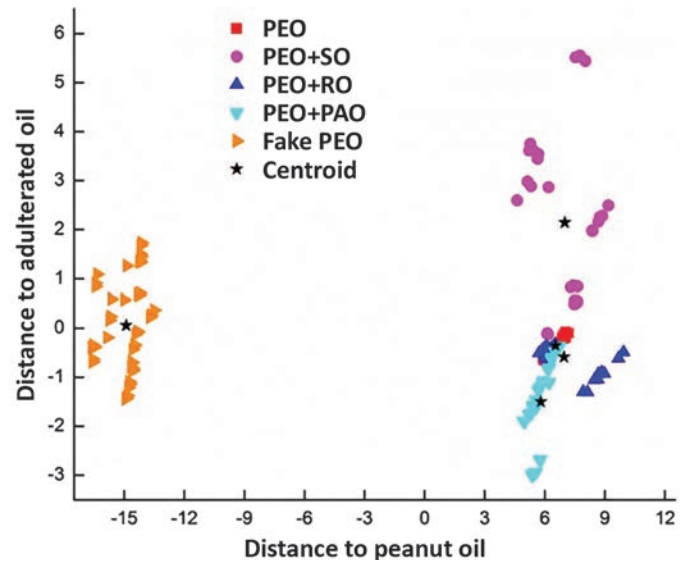


FIG. 3. Discriminant analysis (DA) of pure peanut oil, binary blends (adulteration ratio above 10%), and fake peanut oil (Fake PEO)

oil (PEO) from fake PEO and the adulterated PEO samples when the adulteration ratio is at least 10% of soybean oil (SO), rapeseed oil (RO), or palm oil (PAO), respectively. It is more difficult to separate the binary oil mixtures groups. The results show that different types of the samples are clustered in different regions of the DA score plot (Fig. 3), and higher correct classification rate of 91.7% and 87.5% could be achieved when the adulterant is PAO or SO, respectively, while a low of 79.2% for RO, provided the adulteration ratio is above 30% (Fig. 4).

Therefore, the proposed LF-NMR and chemometrics method can reliably distinguish authentic PEO from counterfeit PEO and the PEO samples adulterated with SO, RO, or PAO. A key feature of this new method is that ^{1}H LF-NMR technique can be used to rapidly and simply detect the adulteration of peanut oils, showing advantages over other time-consuming extraction and purification procedures. Furthermore, it offers great potential for screening the oil species in peanut oil blends "*in situ*."

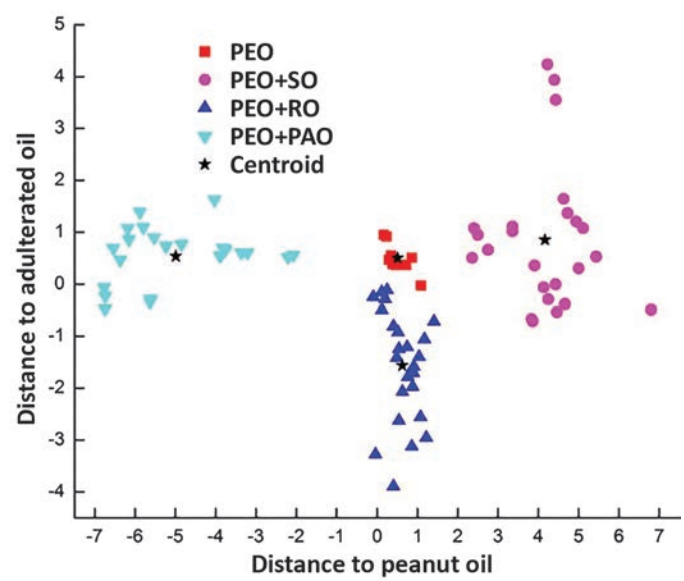


FIG. 4. Discriminant analysis (DA) of peanut oil and the binary blends (adulteration ratio above 30%).

Suggested reading

Zhu, W., X. Wang, and L. Chen, Rapid detection of peanut oil adulteration using low-field nuclear magnetic resonance and chemometrics, *Food Chem*. *216*: 268–274, 2017, http://dx.doi.org/10.1016/j.foodchem.2016.08.051.

Wang Xin is an associate professor at University of Shanghai for Science and Technology (USST), Shanghai, China, where her research typically involves the application of different analytical techniques to solve food safety problems of significance to the rural region in which the university is located, such as establishment of method for evaluating oil quality, the quality prediction of frying oil, rapid determination of physicochemical indicators of oil by Near-Infrared Spectroscopy, relationship between physico-chemical indexes of oil, and LF-NMR characteristics during storage or frying. She can be contacted at 18918629281@126.com.

Zhu Wen-ran is a master's degree student at USST, Shanghai, China, where she concentrates on the detection of peanut oil adulterated with lard, vegetable oil, and waste oil using LF-NMR and chemometrics. Her research project is funded by the National Natural Science Foundation of China, as well as the Key Scientific and Technological Projects of Science and Technology Commission of Shanghai Municipality. So far, the research has resulted in two publications, and has been presented at two national conferences. She can be contacted at zhuwenran2014@126.com.

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Multiple regression analysis to predict palm oil behavior for confectionery applications

Ryan West and Dérick Rousseau

Numerous studies have looked at the crystallization and storage properties of bulk palm oil, but when the crystallization and rheology of bulk palm oil and palm oil mixed with confectioner's sugar were explored over four weeks of storage:

- **high cooling rates matched the solid fat content of palm oil with a less saturated mid-fraction exclusively in the presence of confectioner's sugar and not in bulk systems;**
- **the firmness of palm oil with added confectioner's sugar remained greater than the mid-fraction despite the cooling rate and matching solid fat content;**
- **models were generated by multiple regression analysis to predict solid fat content and firmness of these confectionery oils.**

The structure-functionality relationship of edible fats and oils is impacted by non-fat ingredients, where interactions can alter the fat crystallization pathway. The main ingredient in fat-based confections is often sugar, which facilitates the nucleation of fat crystals [1] in addition to providing sweetness, mouthfeel, and bulk. Fat crystallization is also affected through processing, e.g., formation of numerous, smaller crystals under high cooling rates [2]. The way in which a bulk oil responds to processing may not be retained in the presence of these non-fat ingredients, an issue quite familiar to those who work in industry, which warrants the use of regression models that can predict such behaviors instead of extrapolating from bulk. The effects of volume fraction (ϕ) from confectioner's sugar and cooling rate (q) on the crystallization of palm oil (PO) and its mid-fraction (PMF) are discussed in this article.

PO and PMF are now commonplace in confectionery applications where *trans* fatty acids are restricted. Furthermore, these oils are naturally semi-solid and relatively inexpensive. Due to the tendency for PO and its fractions to crystallize slowly [3], solid fat content (SFC), crystal morphology, and firmness (F) were measured over four weeks. This storage time (t) characterizes the ripening period many palm-

based products undergo prior to distribution. Finally, regression models were generated to predict SFC and F of these oils in response to ϕ, q, and t.

EXPERIMENTAL APPROACHES

Bulk (ϕ = 0.00) PO or a simple blend of PMF (Loders Croklaan, Channahon, Ilinois, USA) with 35 wt.% canola were heated to 60 °C for 30 min in a scraped-surface heat exchanger under shear of 100 rpm and then cooled at either 1, 5, or 10 °C·min^{-1} to a final temperature of 20 °C. Oils were creamed with 6-x confectioner's sugar in a stand mixer before processing to produce oil-sugar blends (ϕ = 0.37). Samples were made in triplicate and measured weekly up to four weeks.

SFC was measured by pulsed nuclear magnetic resonance spectroscopy (Bruker Minispec mq20, Milton, Canada) using the indirect method (AOCS Official Method Cd 16-81) to correct for presence of confectioner's sugar. Six sub-replicate measurements were made for each sample. Confocal laser scanning microscopy (LSM510, Zeiss Inc., Toronto, Canada) at 6300× magnification and stained with fluorol yellow was used to determine crystal morphology. The maximum force as a 45° steel cone penetrated 15 mm into the sample at a speed of 1 mm·s^{-1} using a TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with 30 kg load cell was interpreted as firmness (F).

Multiple regression analysis [4] was used to determine the relationship between SFC or F with ϕ, q, and t. A subset model was selected from an array of third-order polynomials to correct for overfitting using the lowest statistic of Mallows' C_P:

$$C_P = \frac{1}{\sigma^2} RSS_P - n + 2P \qquad (1)$$

where σ^2 is subset error, RSS_P is residual sum of squares, n is number of observations, and P is number of subset parameters [5].

SOLID FAT CONTENT

From the experimental data, subset models of PO (Eq. 2a) and PMF (Eq. 2b) with reasonably explained variance, i.e., R^2, to predict SFC were determined to be:

$$\hat{y}^{SFC}_{\%} = 13.3387 + 15.7514t - 0.2926q^2 - 6.4739t^2 + 0.0270q^3 + 0.8406t^3 + 6.6273\phi q + 0.8183qt - 26.7764\phi^2 t - 0.0293q^2 t - 0.6115\phi q^2 + 1.1521\phi t^2 - 0.1084qt^2 + 0.1851\phi qt \qquad (2a)$$

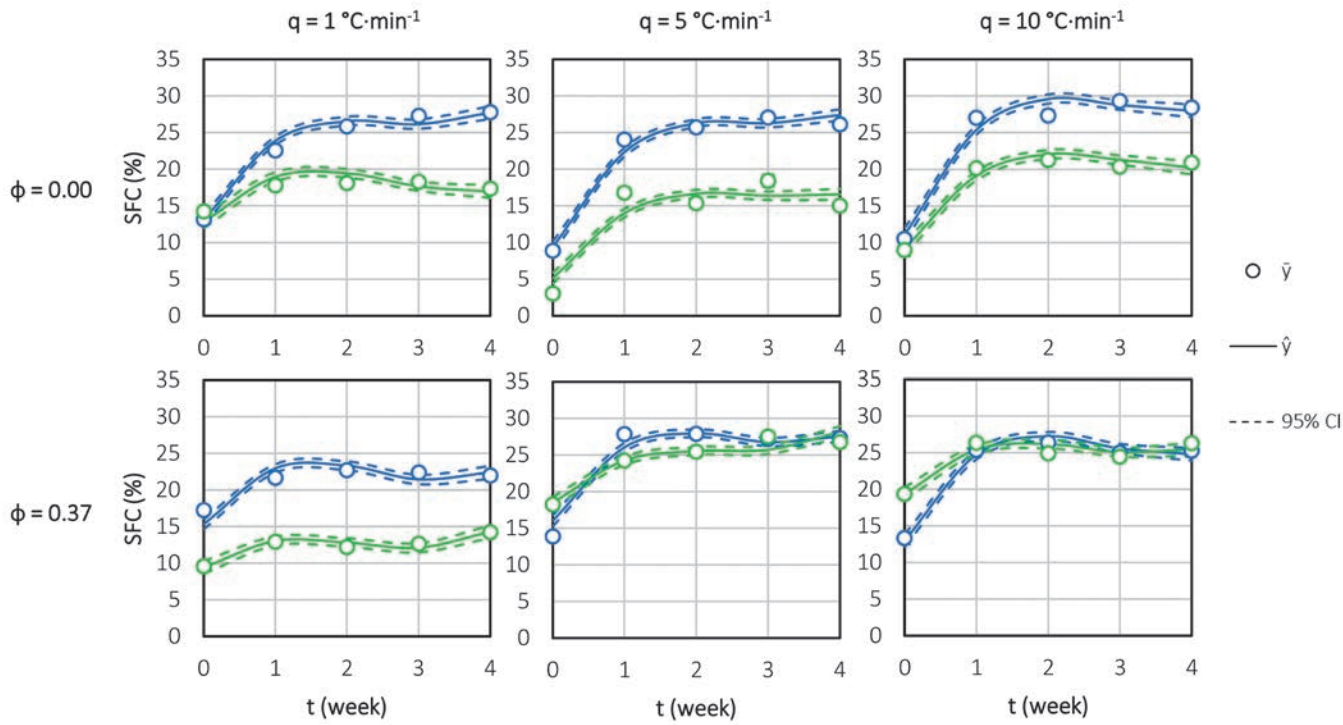
(C_P = 14.69, R^2 = 0.867) and:

$$\hat{y}^{SFC}_{\%} = 16.2772 - 3.7439q + 9.0298t + 0.3033q^2 - 4.4152t^2 - 203.8825\phi^3 + 0.5586t^3 + 1.0856qt + 54.2540\phi^2 q - 23.8221\phi^2 t - 0.0530q^2 t - 1.4525\phi q^2 + 2.4364\phi t^2 - 0.0775qt^2 - 0.4293\phi qt \qquad (2b)$$

(C_P = 15.00, R^2 = 0.879), respectively. Predicted SFCs ($\hat{y}$) with 95% confidence intervals (CIs) are depicted in Figure 1 (page 18) in addition to experimental means ($\bar{y}$) with standard error. PO was on average higher in SFC than PMF due to being 1.5 and 15% higher in diacylglycerols and palmitic-rich triacylglycerols [1]. Fewer acylglycerols are required to form a stable nucleus on catalytic foreign surfaces which reduces the induction time of nucleation [2]. The general increase of SFC at 0 weeks by addition of confectioner's sugar indicated enhanced crystallization which supports this notion.

SFC increased dramatically as q increased from 1 to either 5 or 10 °C·min^{-1} in the oil-sugar blends whereas bulk oils were less responsive to q. The greater sensitivity of PMF-sugar to q which allowed it to match PO-sugar in SFC by week

FIG. 1. Effect of sugar volume fraction (ϕ), cooling rate (q), and storage time (t) on experimental (ȳ) and predicted (ŷ) solid fat content (SFC) with 95% confidence interval (CI) for PO (Eq. 2a, blue) and PMF (Eq. 2b, green)



4 highlights the importance of processing in generation confectionery products with reduced saturation. The SFC of PMF-sugar increased by 188 and 185% by the end of storage as q increased to 5 and 10 °C·min^{-1}, respectively, while PO-sugar had increased by 124 and 115%. The way in which the oil-sugar blends responded to q in comparison to their bulk counterparts brings to question the accuracy of data that is extracted from bulk systems where crystallization is unobstructed.

MORPHOLOGY

The impact of ϕ and q on fat crystal morphology of PO and PMF by week 4 are depicted in Figures 2 and 3, respectively. Both oils possessed clustered spheroidal crystals ≤ 15 µm in diameter with fine needle-like network crystals at a q of 1 °C·min^{-1}. The number of crystals increased with increasing q immediately following processing in both bulk oils, however, they were slightly smaller in diameter and clustering had diminished. Furthermore, long, bladed crystals ≤ 40 µm in length formed in parallel to the fine network crystals upon storage in bulk PO at higher q (Fig. 2). These bladed crystals were notably absent in bulk PMF using the same processing conditions (Fig. 3).

Smooth, obloid crystals ≤ 11 µm in diameter formed immediately following processing at a q of 1 °C·min^{-1} in the presence of confectioner's sugar for either oil and remained throughout storage (Figs. 2 and 3). These crystals were smaller compared

FIG. 2. Effect of sugar volume fraction (ϕ) and cooling rate (q) on crystal morphology for PO at week 4 (bar = 20 µm)

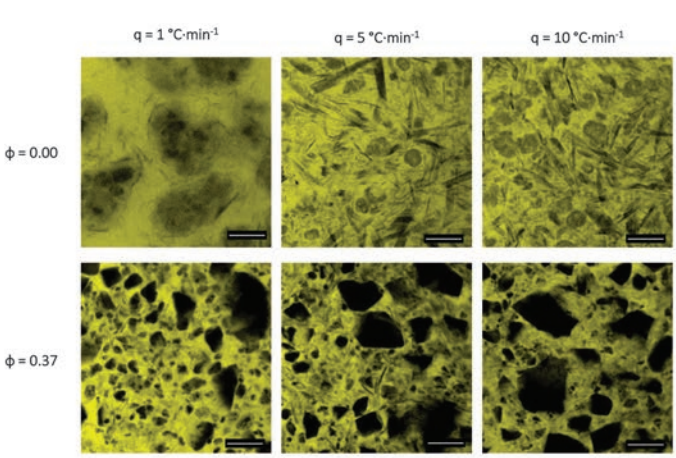


FIG. 3. Effect of sugar volume fraction (ϕ) and cooling rate (q) on crystal morphology for PMF at week 4 (bar = 20 µm)

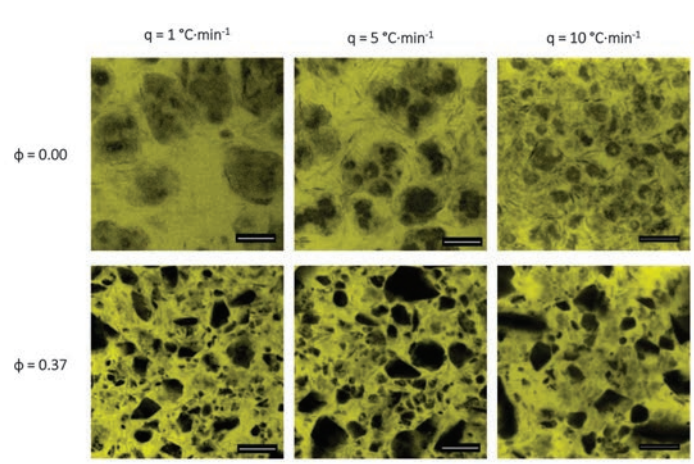
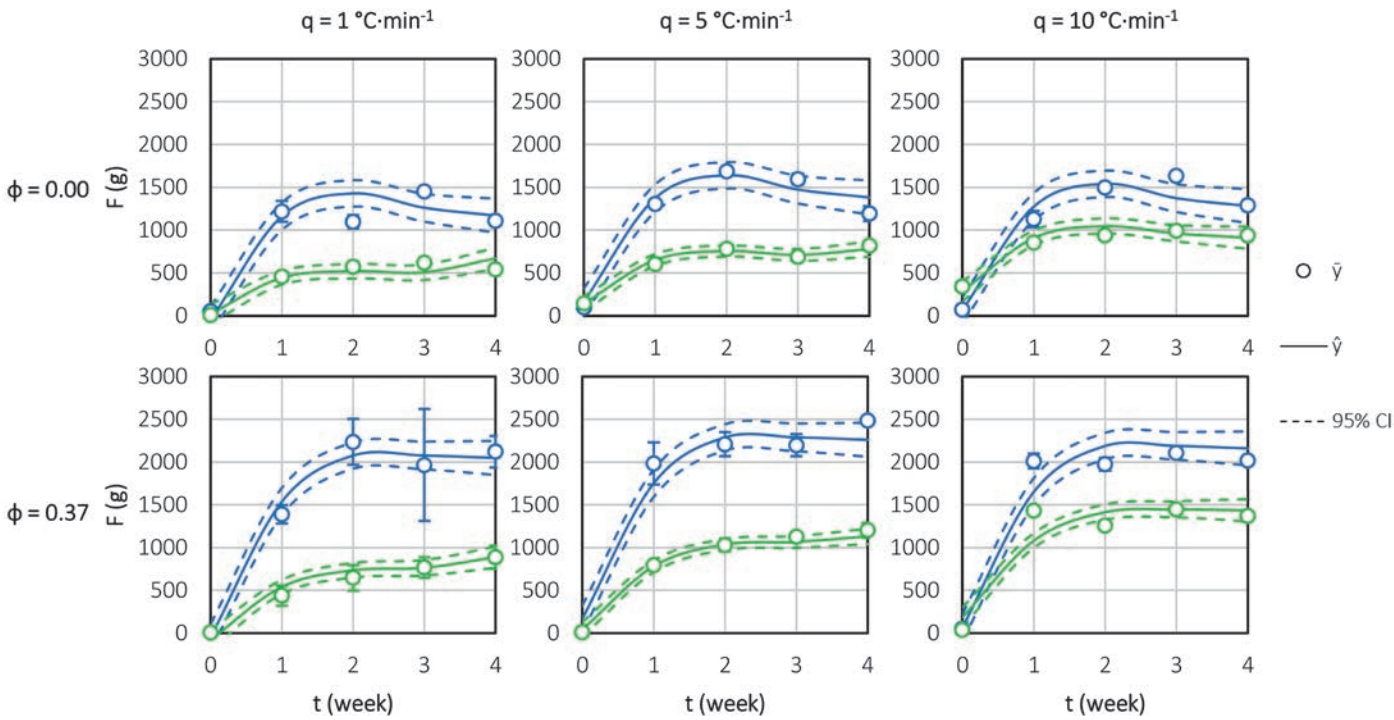


FIG. 4. Effect of sugar volume fraction (ϕ), cooling rate (q), and storage time (t) on experimental (ȳ) and predicted (ŷ) firmness (F) with 95% confidence interval (CI) for PO (Eq. 3a, blue) and PMF (Eq. 3b, green)



to their bulk counterparts and contained particulate inclusions from the sugar. Needle-like network crystals formed upon storage although the haze casted when oil-sugar blends had a q of 10 °C·min^{-1} may suggest crystal size had reduced beyond the detection threshold of the microscope, i.e., < 0.5 µm.

FIRMNESS

Large deformation tests are used to obtain textural properties of foods, e.g., F, in either the absence of or conjunction with a trained sensory panel. The subset models of PO (Eq. 3a) and PMF (Eq. 3b) to predict F were determined to be:

$$\hat{y}_g^F=-141.6610+101.3698q+1849.3576t-8.0682q^2-725.3517t^2+84.7593t^3+1154.5504\phi t-140.3555\phi t^2 \quad (3a)$$

(C_P = 4.29, R^2 = 0.885) and:

$$\hat{y}_g^F=-5.6982-278.1098\phi+29.1745q+651.3142t-295.3342t^2+43.2731t^3+614.3112\phi t+29.9731qt-105.4934\phi t^2-7.6431qt^2+22.9902\phi qt \quad (3b)$$

(C_P = 9.70, R^2 = 0.910), respectively, which are depicted with 95% CI in Figure 4 in addition to experimental means (ȳ) with standard error. PO was more firm than PMF on average due to its acylglycerol composition [1] and higher SFC (Fig. 1). The increase in F from bulk after addition of confectioner's sugar was greater in PO than PMF (Fig. 4). Despite matching SFCs in both oil-sugar blends by week 4 after increased q, PO-sugar remained consistently higher in F throughout storage than PMF-sugar. PMF-sugar did increase in F after increased q, however, it remained at least 650 g less firm than PO-sugar by the end of storage, a difference that would surely be detectable to

Further reading

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[5] Mallows, C.L. (1973). Some Comments on C_p. *Technometrics 15*: 661–675.

consumers. Further factors such as the relationship between morphology and large deformation, presence of multiple phases, and specific interactions between fat crystals and confectioner's sugar [2] must be taken into account when constructing models through multiple regression analysis to better understand these discrepancies.

MORE TO COME

In this article, we have highlighted through empirical data the limitations of extrapolating information from bulk oils to determine essential properties, such as SFC and F, of confectionery systems. Subset models where one can predict with accuracy these properties if ϕ, q, and t are known were generated via multiple regression analysis to rectify this shortcoming. In the long run, it is our intention to showcase multiple regression analysis as an industrial tool where palm-based confections are created.

Ryan West is a doctoral candidate from Ryerson University in Toronto, Canada, where he models fat crystallization behavior in confectionery systems. He also received a Professional Chocolatier Certificate from the Center for Hospitality and Culinary Arts at George Brown College. Ryan can be contacted at ryanwest@ryerson.ca.

Dérick Rousseau has been a professor of food science at Ryerson University in Toronto, Canada, since 1998. There, his main research focuses on formation and stability of colloidal systems and exploring ways to improve their functionality. Over the years, he has held numerous collaborations with international industrial partners on processed foods, crude oil, and personal care products. Dérick can be contacted at rousseau@ryerson.ca.

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Simplified, highly multiplex pathogen analysis for agricultural, food, water, and environmental sampling

Rick Eggers, Melissa May, Kevin O'Brien, Milan Patel, Carl Yamashiro and Michael Hogan

- Safety monitoring of medicines, food, and the environment have become more challenging due to an increased number of pathogens and a shorter timeline for testing. Global trade has intensified the need to identify regional pathogens from various regions of the world, and transporting products over increased distances has condensed the timeline for selling products with limited shelf lives.
- Today, testing for pathogens is primarily done through microbiological culturing methods. Such methods take days for results to be obtained, and are not very amenable to testing large quantities of samples against multiple pathogens.
- This article discusses the advantages and disadvantages of various pathogen testing solutions and describes a new rapid pathogen detection method with multi-analyte capabilities.

The effectiveness of pathogen testing is assessed based on seven critical parameters: specificity, sensitivity, quantification, sample preparation, multiplexing, time to result, and cost. Definitions of these parameters are provided in Table 1. Ideally, a pathogen test should exhibit high sensitivity and specificity, effective quantification covering the relevant dynamic range, sample preparation that is rapid and effective for all sample matrices encountered, and as short a time to result as possible, preferably less than 24 hours. Following is a discussion of the pros and cons of several pathogen testing solutions based on the seven critical parameters.

STANDARD MICROBIOLOGICAL CULTURE

Pros: For nearly a century, plate-based analytical microbiology has been the gold standard for medical, food, and agricultural testing. It is relatively inexpensive in terms of equipment cost, and has fostered a comprehensive set of culture-based technologies and analytical methods to

support it. The primary data to be derived from plate-based culture, namely the detection of viable microbes at the single organism limit, has defined the theoretical limit of live pathogen detection.

Cons: Specificity can be suboptimal since plate-based methods are very empirical in nature and based on identification through colony morphology on growth-specific media. Sensitivity can be compromised, as there can be selective bias due to some media not being ideal for certain organisms or some of their variants. Plate-based methods also suffer from the currently accepted bias that all living microbes are readily cultured, when it has been repeatedly observed that microorganisms can be viable but non-culturable, thus leading to potential false negatives. Absolute confirmation of colony identity requires secondary analysis via antibody-based or nucleic acid-based testing of the protein or deoxyribonucleic acid (DNA) complement of a colony. Altogether, time to results can be very long. For slow-growing organisms, it can take days or even a week to obtain culture results and confirmatory testing.

MOLECULAR METHODS

Polymerase chain reaction (PCR)—DNA and RNA

In PCR, a well-defined DNA sequence is selectively copied, enzymatically, to produce as many as 1 billion identical copies of the original DNA sequence. In the case of pathogen testing, the DNA region is typically a sequence that is about 100–200 base pairs long and unique to the pathogen of interest. In particular, a variant of PCR called quantitative real time PCR (qPCR) has evolved to become the standard for DNA-based pathogen testing of food, agriculture, water, and environmental samples. The availability of DNA sequence data for many microbial genomes makes it possible to rapidly design highly specific and sensitive qPCR-based tests for nearly all microbial pathogens.

Pros (qPCR): Generally speaking, the qPCR technology is highly specific. Its specificity is derived from the use of two primers—and often an internal probe—which are specific for the analyte of interest. Sensitivity of qPCR is excellent, and time to result is more rapid than with culture-based tests

TABLE 1. Parameters for pathogen test assessments

Parameter	Definition
Specificity	The ability to unambiguously identify specific pathogens, including many in parallel
Sensitivity	The ability to detect pathogens as a specified level, including at the limit of 1 colony forming unit (CFU) per sampling event
Quantification	The ability to quantify pathogens at the limit where counting statistics are limited by the inherently stochastic property of single CFU events per sampling event
Sample preparation	The ability to accommodate both unprocessed materials and highly processed materials (e.g., cooked foods, organic extracts) efficiently and effectively
Multiplexing	The ability to detect a panel of multiple pathogens, in parallel, all in the same test
Time to result	The time it takes from the start of sample preparation to obtaining analytical results from the test data obtained
Cost	Total cost of reagents, equipment, and personnel to perform the test

since qPCR takes a few hours to perform versus days for a culture-based test.

Cons (qPCR): Despite many strengths, qPCR is difficult to multiplex, with each qPCR reaction being specific for one or two pathogens only. The very large panel of qPCR tests that would result from the concurrent analysis of 1–2 dozen pathogens would be costly and not ideal for high throughput. "Clean" samples must be prepared since certain contaminants can inhibit the PCR or interfere with the fluorescence detection, making this parameter more challenging and possibly increasing costs and time to result.

Microarrays

In the nucleic acid testing arena, it has been known for two decades that microarray technology constitutes the gold standard for specific, highly multiplexed nucleic analysis. The microarray technology is based upon the fabrication of a microarray (i.e., a micro-scale matrix of nucleic acid probes linked to a surface, such as a microscope slide) containing 100–10,000 nucleic acid probes that can be mass-produced.

In the context of microbial analysis, each surface-associated nucleic acid probe (20–30 bases long) is designed to be specific for a DNA sequence unique to a specific pathogen. Thus a 10x10 element microarray could interrogate up to 100 sequence analytes in parallel, via fluid phase hybridization of a DNA containing sample to the surface.

Pros: Microarray technology can query many pathogens in parallel. Historically, microarrays have been expensive to fabricate; however, the cost per microbial test in a large microarray panel is much lower than the cost for a single qPCR assay. Microarrays are much less sensitive to sample contamination which can interfere with qPCR fluorescence detection and measurement. Microarray analysis is dependent on fluid phase analyte concentration, thus microarray testing can deliver quantitative analysis among the many of tests performed in parallel on the microarray. Note there is 6-log dynamic range achievable with qPCR analysis of a single analyte, while microarrays have a 2–3 log range sufficient for most types of microbial testing.

Finally, microarray analysis has, built into it, the ability to resolve extremely complex DNA mixtures in a way that is nearly impossible for other methods of DNA analysis, such as qPCR or DNA sequencing. This unique capability is based on the fact that the interaction of a fluid phase DNA sequence element (such as a 20–30 base region of a specific pathogen) with its surface-bound cognate probe can be spread out in 2-dimensions on the microarray surface. Thus, whereas a complex mixture of 1-2 dozen pathogen DNAs might generate superimposed DNA that is hard to disengage via qPCR, each solution-surface interaction occurs at a unique, pre-determined location on the microarray, generating its own, isolated DNA-binding signal that can be detected and quantified spatially via routine imaging of target-probe binding.

Cons: Historically, microarray analysis has been viewed as being too expensive for routine microbiological screening, but recent advances are changing that perception. Thus, although the first research-scale deployment of microarray technology in the area of microbial testing via microarrays was published nearly 20 years ago, the technology has only been widely accepted as a high-end research tool.

FIG. 1. a. PDx-C 12 well microarray

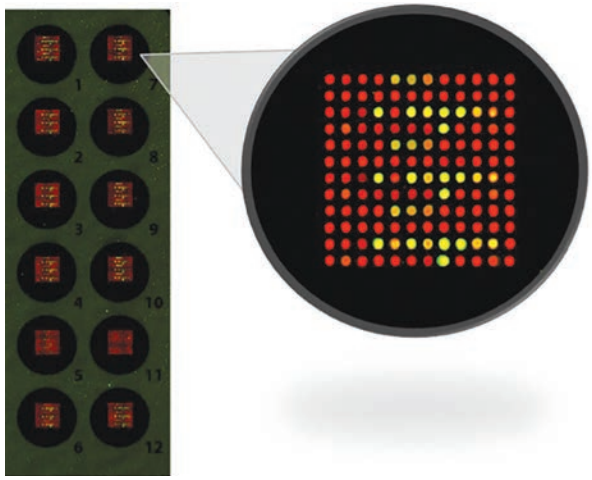
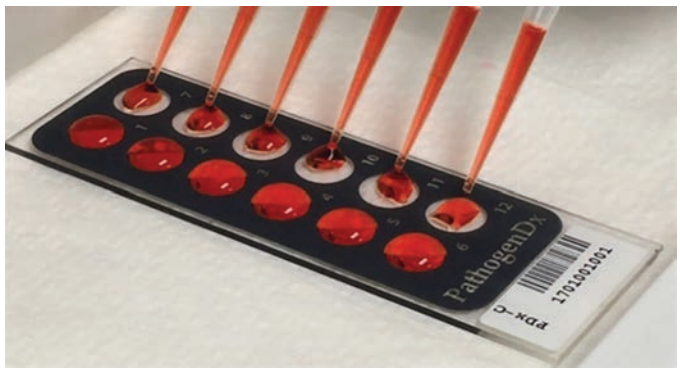


FIG. 1. b. Open architecture



A low-cost, low-tech implementation of microarray technology

Based on the desire to deliver a highly-multiplexed microarray test to the microbial testing market, we have sought to add new technology to microarray design and to use that new technology to build a highly-multiplexed microarray technology that is as sensitive and specific as qPCR, but less expensive (especially when measuring a large number of pathogens as a panel) due to the new technology's high capacity for multiplexing for routine analytical use. This **PDX-C Microarray Test** offers several distinct advantages over conventional microarray technology.

Simplified microarray manufacture and use. Simplifying technologies fall into two broad classes.

The first simplifying core technology is a version of microarray manufacture that allows ordinary DNA oligonucleotides (fabricated without chemical modification) to be printed directly onto standard glass slides to form panels of microarrays (12 each per slide), which are fluidically isolated via a hydrophobic barrier coating (Fig. 1. a). This patent-protected approach reduces the cost of microarray manufacture by about a factor of 10 and produces medium density microarrays, such as the standard 12x12 format in Figure 1. a., at a cost that is comparable to the reagent cost for a single qPCR reaction.

A second attribute of this simplified microarray technology is that the oligonucleotide probes are linked to the underlying surface in a configuration that allows the probe to hybridize to solution state target DNA ***at lab ambient temperature***, while retaining single nucleotide base-pairing discrimination. Having achieved that second simplification, samples can be applied to microarrays via ordinary manual or automated pipetting while retaining a simple "open" architecture throughout (Fig. 1. b.), much as ordinary microtiter plates are used for immunoassays of the kind used widely in serology for 40 years.

Simplified Sample Processing. As is the case for most microarrays, the PDx-C microarray test is initiated by a PCR reaction which serves to amplify and dye-label specific microbial DNA segments. What is unique to the patented PDx-C technology is that the PCR is orchestrated as a sequential pair of reactions to be performed on each specimen: The first reaction amplifies the DNA regions of interest, and the second PCR reaction used to label those regions with a fluorescent dye is detected by 2D image analysis, subsequent to binding to the microarray.

Glossary

Deoxyribonucleic acid (DNA)—Deoxyribonucleic acid is a molecule that carries the genetic instructions used in the growth, development, functioning, and reproduction of all known living organisms and many viruses.

Polymerase chain reaction (PCR)—a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

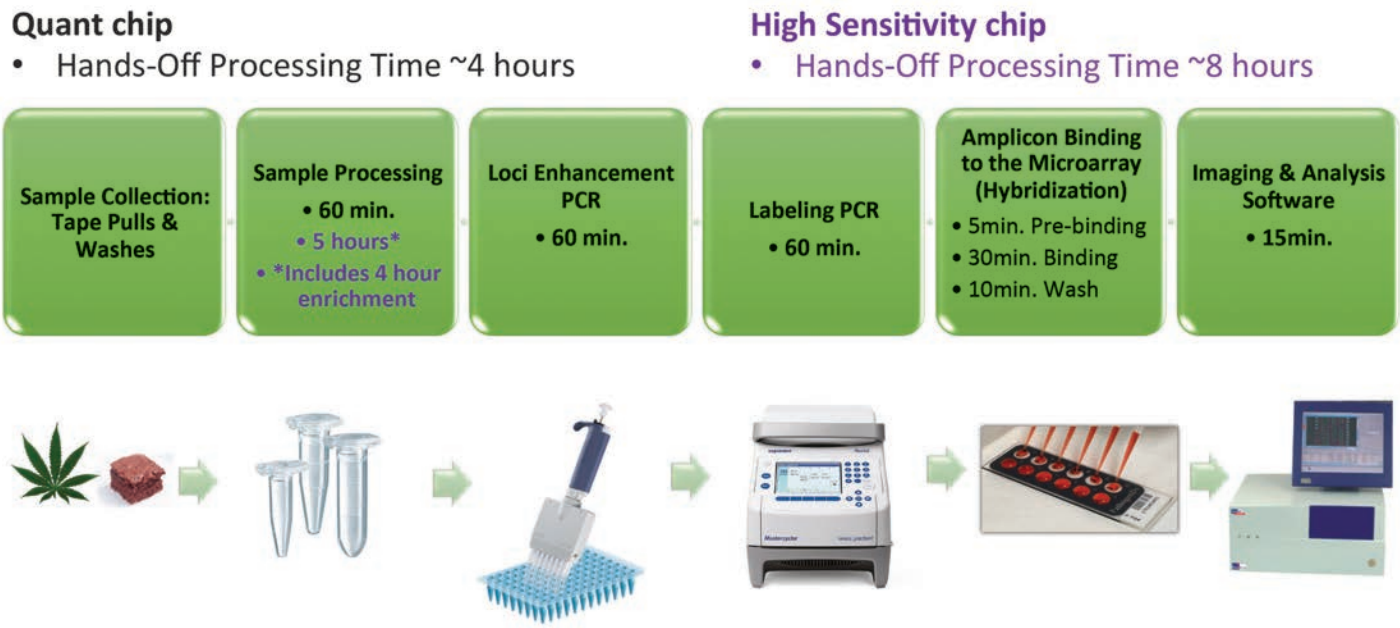
Enzyme-linked immunosorbent assay (ELISA)—is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology.

Microarray—(also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome

PDx-C—a patented technology to rapidly detect and quantify simultaneous, multiplex analysis of relatively large panels of bacteria, yeast and mold on recreational or medicinal cannabis

Multiplex and multi-analyte testing—testing hundreds of food, medicinal, or environmental samples at the same while analyzing multiple pathogens simultaneously

FIG. 2. PDx-C process flow with less than six hours turnaround



This is referred to as "Raw Sample Genotyping" (RSG) in which this tandem PCR approach to generate DNA is introduced into a PCR reaction without DNA purification.

A representative microarray workflow developed for cannabis testing, based on such Raw Sample Genotyping (RSG) is displayed in Figure 2. Plant material is simply washed in water to displace surface-associated microbes into fluid suspension. The microbes are harvested from the suspension by bench-top centrifugation to form a pellet, which is then lysed by addition of an enzyme cocktail to release the DNA from the bacteria and eukaryotes in the pellet. About 10% of the lysed pellet is then used as sample input into the two-step PCR reaction. Subsequent to the PCR steps, the entire PCR product is then applied as-is to the microarray for room temperature hybridization analysis.

By eliminating the work and kit costs associated with DNA purification while keeping the smallest possible total sample volume, it has been observed that this combination of RSG and microarray technology not only reduces the time and effort required for such testing by more than a factor of two, but it also allows a relatively large fraction of the input sample to be measured—a capability that has proven to be especially important when the number of pathogens per sample is reduced to the 1 CFU limit.

TABLE 2. PDx-C twice as sensitive in detecting 1 CFU than plate culture methods

PDx Array Results	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Replicate 8	Replicate 9	Replicate 10	No Matrix Control	Spiked Unenriched Total
E. coli	-	-	-	+	++	+	++	+	+	+	-	7/10
Salmonella enterica	-	-	-	-	-	+	-	-	+	-	-	2/10
Aspergillus flavus	+	+++	-	+++	-	-	-	++	-	-	-	4/10
Aspergillus niger	+	+	-	+	-	-	++	+	-	-	-	5/10
Aspergillus fumigatus	+	+++	+++	-	-	-	-	+++	-	-	-	4/10
Plating CFU Results												
MacConkey (bacterial)	-	-	-	2	1	-	-	-	-	-	-	2/10
Sab Dex (fungal)	-	1	-	1	1	-	-	-	1	-	-	4/10
E. coli	-	-	-	1	1	1	-	-	-	-	-	3/10
Salmonella enterica	-	-	-	1	-	-	1	-	-	-	-	2/10
Aspergillus flavus	-	-	-	1	-	-	-	-	-	-	-	1/10
Aspergillus niger	-	-	-	-	1	-	-	-	-	-	-	1/10
Aspergillus fumigatus	-	1	-	-	-	-	-	-	1	-	-	2/10

+++	40,000 – 65,000 RFU
++	20,000 – 39,999 RFU
+	1,000 – 19,999 RFU
-	0 – 999 RFU

No Enrichment

PDX-C Detection Rate = @2X Plate Count Detection Rate

Driven by Copy Number

Applications Suitable for RSG + Microarray Analysis. Food, natural product, and environmental screening has begun to focus on simultaneous, multiplex analysis of relatively large panels of bacteria, yeast, and mold. The first implementation of PDx-C was focused on cannabis compliance testing of medical and recreational marijuana, which are regulated for pathogen contamination by the local state. This microarray-based cannabis test, referred to as PDx-C has been subjected to exhaustive testing relative to plate- based cultured methods by Steep Hill Labs in Berkeley, CA (data to be published elsewhere).

Results of such cannabis testing have been submitted to the States of Alaska and Hawaii to support the use of the PDx-C test as the primary method of pathogen testing. The general conclusions drawn were that the PDx-C test has sensitivity and specificity equivalent to that of plate based culture, in the limit of 1 CFU per sample, for both bacterial and yeast contamination, with a much shorter time to result. A summary of those data is presented as Table 2.

In conclusion, it is clear that microbial testing is moving towards a situation where DNA-based molecular testing is becoming the new gold standard. However, as the need for multiple pathogen measurement and species differentiation continues to grow, a different platform will be required to provide faster throughput, robust test content via routine, high-level multiplexing, and the resulting economies of scale. Food safety, agriculture, water, and botanical testing will all greatly benefit by shifting their microbial testing method from conventional practices to a DNA microarray-based platform, such as PathogenDx, that allows large-scale microbial analysis to be conducted as a single test. It is envisioned that testing for microbe panels as large as 1–2 dozen bacteria and fungi will become routine for products as diverse as oilseeds, peanut butter, dairy products and meat as it becomes more widely recognized that these food substrates can each foster the growth of multiple, highly toxic pathogens.

Rick Eggers is a Senior Scientist at PathogenDx in Tucson, Arizona, USA. He can be contacted at reggers@pathogendx.com.

Melissa May is a Scientist at PathogenDx in Tucson, Arizona, USA. She can be contacted at mmay@pathogendx.com.

Kevin O'Brien is a Senior Scientist at PathogenDx in Tucson, Arizona, USA. He can be contacted at kobrien@pathogendx.com.

Milan Patel is the chief operating officer at PathogenDx in Scottsdale, Arizona, USA. He can be contacted at mpatel@pathogendx.com.

Carl Yamashiro is VP Product Development at PathogenDx in Scottsdale, Arizona, USA. He can be contacted at cyamashiro@pathogendx.com.

Michael Hogan is CSO at PathogenDx in Tucson, Arizona, USA. He can be contacted at mhogan@pathogendx.com.

Sustainable extraction of nature's oil and protein storage vesicles

James V. Gruber

- Conventional commercial oil isolation processes squeeze oil from oilseed storage structures, called oleosomes, and harsh organic solvents are often used to enhance oil recovery.
- Alternatively, oilseed oleosomes can be commercially recovered in their natural state, using only water under mild conditions.
- The commercial use of oleosomes has expanded significantly in the cosmetics industry, where they serve as a natural and sustainable substitute for many synthetically derived emulsions.
- This article explains how oleosomes are isolated commercially, and how this process differs from standard oil recovery operations. It also reviews current uses for oleosomes in cosmetic ingredients.

It is generally recognized that nature excels at manufacturing items of such intricacy that, as humans, we often marvel at them. In particular, living cells are extremely efficient at packing significant amounts of materials into very small spaces. Examples include deoxyribonucleic (DNA) packing into the cellular nucleus, starch packing into starch granules, and oil packing in oilseed oleosomes. Oilseeds, like safflower, sunflower, hemp, canola, and the like, store oil in small encapsulation vesicles called oleosomes. Generally speaking, oleosomes are triglyceride oils (unique for each plant) surrounded by a phospholipid shell that is enshrouded in a monolayer sphere of very unique proteins called oleosin proteins. In-depth discussion on the structure of the oil seed oleosome can be found in a review article by (Tzen, J.T.C., Integral proteins in plant oil bodies, *ISRN Botany*, 2012, https://www.hindawi.com/journals/isrn/2012/173954/).

The oleosomes are expressed in the endoplasmic reticulum of the oilseed, where they maintain the oil within the seeds in a very stable state until this oil is required for germination and growth of the plant. In addition, protein storehouses in each seed store proteins that are brought forth

when the seed germinates to provide a nitrogen source for the small plant. For centuries, humans have relied on oils from seeds to provide nutrition and cooking benefits. Oils from various plants are also popular topical treatments, as they help improve skin barrier function, hydration, and conditioning.

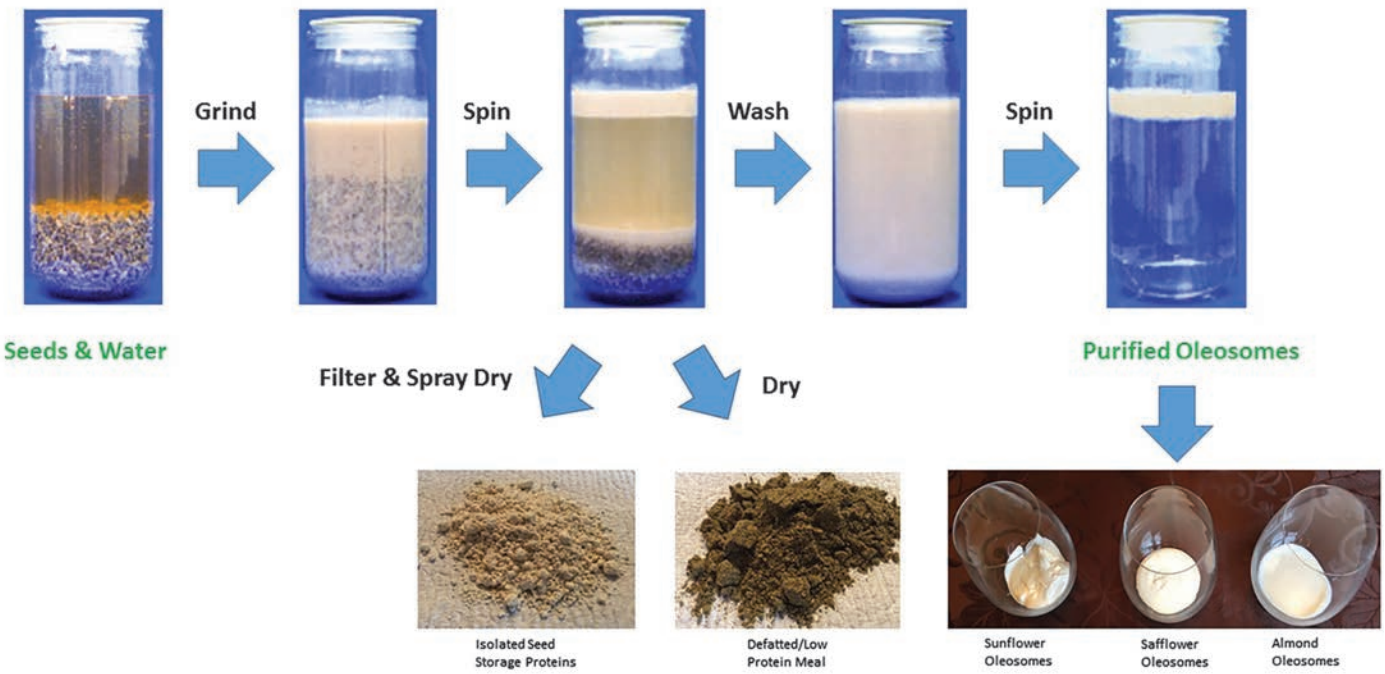
Isolation of oilseed oils has evolved through the years, and today oil seeds can be extracted to provide purified oils that are sold regularly in huge quantities around the world. YouTube, videos of the processes to extract sunflower oil (https://www.youtube.com/watch?v=VZZuu5ROcdQ) and canola oil (https://www.youtube.com/watch?v=Cfk2IXlZdbI) can be easily found. Typically, oil seeds are run through a screw extruder, which crushes the seeds under high pressures and temperatures. Crushing this way ruptures the oilseed oleosomes to release their oil. Pressing extraction creates a seed cake which still typically contains about 25–30% of the available oilseed oil. To make the extraction process truly efficient, the seed cake must be further extracted using an organic solvent. Typically, the petroleum-based solvent hexane is used to remove the remaining oil in high-temperature extractors. Because hexane is toxic, the extracted oil must be vacuum-evaporated to remove any hexane residues. Then, the extracted oil is combined with the pressed oil to create a crude oil extract, which is further refined and sold as edible oil.

During crushing and solvent extraction, the storage proteins in the seed are effectively crushed, denatured, and trapped in the hulls and fibrous components. The resulting cake, or seed meal, undergoes an additional vacuum-evaporation to remove any hexane residues, and is typically sold as animal feed. Hence, much of the protein's value is lost during extraction, and ultimately diminished by its end use, as protein consumption via animals is not the most efficient use of plant proteins.

THE BOTANECO PROCESS: A GREENER WAY TO OBTAIN OIL AND PROTEINS

The isolation of oilseed oleosomes from oilseeds is well-established and has been done on a small scale (typically laboratory scale) for years. However, commercial application of plant oil seed oleosomes has only been going on since about 2008, when Botaneco introduced these unique ingredients into commercial production and sale. Since then, the use of oleosomes in personal care applications has grown. As seen in Figure 1, the extracted oleosomes do not appear as a clear liquid, but resemble a creamy emulsion, much like mayonnaise.

FIG. 1. Pictorial summary of Botaneco's oilseed isolation processes and individual product streams, including storage proteins, defatted/low protein meal, and oleosomes (sunflower, safflower, and almond oleosomes)



To date, Botaneco has primarily focused its commercial efforts on isolating safflower oleosomes. The safflower seed is an ideal seed for the development of highly sustainable oleosomes, as it grows without genetic modification in low-water environments. But other seeds, such as those of sunflower, almond, hemp, and canola, contain oleosomes that can be extracted using the Botaneco processes. The company continues to look at other oil-bearing seeds to grow its portfolio of oilseed ingredients.

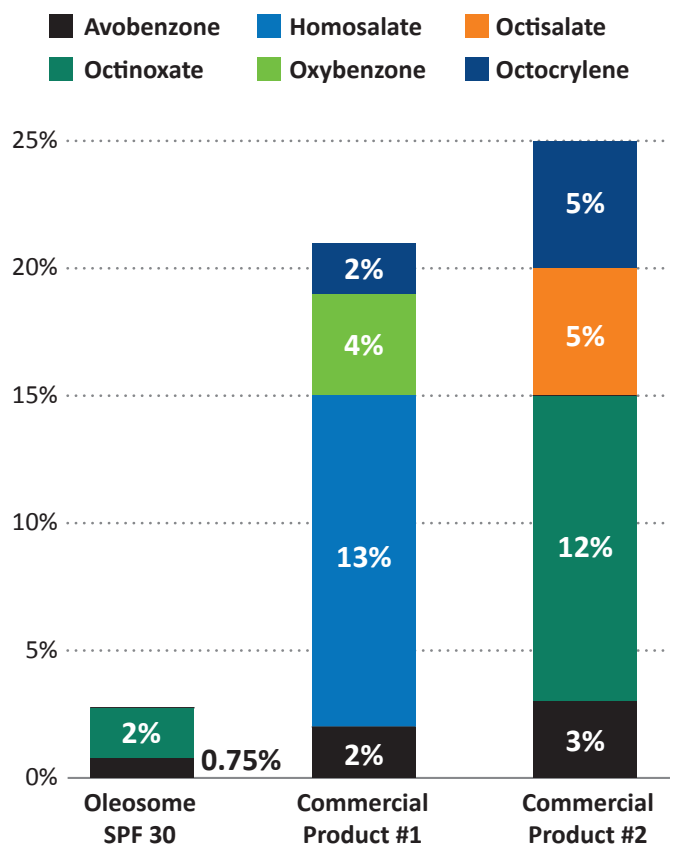
In 2013, Botaneco became an independent, privately-held company. At that time, the company invested significantly in automating and improving its oleosome extraction process. The process of oleosome extraction which is done regularly now at Botaneco, begins like all oil seed oil extractions, with the oilseeds. In the case of safflower oleosome extraction, the seeds are washed with water and then passed through a series of gentle grinding steps to create an aqueous seed extract. What is interesting about the Botaneco oleosome isolation process is that it is all done with small amounts of water under very mild conditions. Temperatures never exceed 60°C. Most importantly, unlike conventional oil extraction, the oleosome extraction process does not use hexane or other organic solvents. Extraction of oilseed oleosomes occurs only using water as the extracting medium.

The seed extract that results from this initial extraction is further refined with a series of washing steps that still use only water. The oleosome fraction rises naturally during the manufacturing process due to density differences between the oil phase (oleosomes) and the water phase. Through a series of further decanting and centrifuging steps, highly refined oleosomes containing less than 2% of residual seed proteins can be made.

Because the Botaneco extraction methods use only gentle grinding and water extraction, the process creates three plant streams: the oil fraction containing the cleaned and stabilized oleosomes, an aqueous phase, and a small residual solid phase that primarily contains the cellulosic and hemicellulosic materials from the hulls. The aqueous phase is of particular interest, as it contains a very high yield of the oilseed storage proteins. In contrast, during conventional extraction processing with heat, pressure and solvents, the storage proteins are lost with the fibrous hulls and can only be recovered when extreme extraction conditions are applied—and even then, yields are very low. What's more, the proteins extracted from pressed oil seeds are fractured and denatured, which dilutes their effectiveness—particularly in nutritional applications.

The isolation of plant storage proteins has begun in earnest at Botaneco, and the company now supplies proteins isolated from safflower seeds to the personal care industry [INCI Name: *Carthamus tinctorius* (Safflower) Seed Extract]. The Botaneco extraction processes is unique because it allows the company to isolate very clean oleosomes and highly refined plant storage proteins in a single manufacturing process. This cannot be accomplished using any other known oilseed extraction technologies, including supercritical CO_2 extraction (which would likely destroy the oilseed oleosomes as well). In addition, the resulting meal that comes from the manufacturing plant stream can still be used as an animal feed, which means that the entire seed is used in a way that extracts the most valuable components (oil and protein) while maintaining the meal for animal nutrition. Plus, the oil and meal do not require solvent stripping, which involves high temperatures or vacuum—both of which are very aggressive.

FIG. 2. Percentage of sun care active levels of an oleosome sun care formulation containing two well-known actives, clinically tested at SPF 30 on 10 people, compared to two commercial sun care products found on the market that make similar SPF 30 claims



COMMERCIAL COSMETIC USES OF SAFFLOWER OLEOSOMES

Oleosomes from Botaneco's oilseed extraction processes are finding uses in personal care, where they function as natural emulsifiers, humectants, conditioning agents, and barrier-improving ingredients. To date, commercial products containing safflower oleosomes [INCI Name: *Carthamus tinctorius* (safflower) oleosomes] can be easily purchased on the web. They have found applications in numerous skin and hair care products. Because they are made from non-GMO sourced seeds and isolated in a very natural way from the seed, they are a popular ingredient in products requiring gentleness, such as sun care products for babies and children.

Safflower oleosomes are unique because they contain measureable amounts of natural vitamin E which the plant places into the oleosomes to maintain the integrity of the oil—

even during times of drought. In conventional extraction processes, this natural vitamin E may remain in the oil, but the isolated oil is exposed to significant amounts of oxygen. In contrast, intact oleosomes serve as a barrier to oxygen, so the oil contained in them is less susceptible to oxidation and rancidity. Oil-based actives can be loaded into the oleosomes as well, which further enhances their benefits in formulations. Recently, Botaneco launched a new oleosome-based ingredient [INCI Name: *Carthamus tinctorius* (safflower) oleosomes (and) *Carthamus tinctorius* (safflower) seedcake extract] that has clinically demonstrated benefits for improving the effectiveness of organic sunscreens. In clinical studies conducted by the company, it was found that loading two sunscreen actives (Avobenzone and Ethylhexylmethoxycinnamate) into the oleosome-based ingredient allows the active sunscreen agents in a formulation to be reduced by nearly 80%. Figure 2 shows the amount of active sunscreen required to attain an SPF of 30 (measured clinically on 10 humans) vs two known commercial products presently on the market. The reduction in the amounts of active sunscreen agents needed to maintain sunscreen benefits is dramatic.

Consumers continue to look for products that contain naturally derived ingredients. This consumer preference is active in the nutrition, food, and cosmetic markets. Botaneco has worked to develop highly sustainable oil seed extraction technologies that create unique ingredients that are finding uses in personal care and food. By extracting the oil seeds using only water and mechanical processes, the company is able to create multiple plant streams of oleosomes, seed proteins and meal that are unique and offer new and exciting opportunities for continued develops in these industries. Most recently, the company launched a technology for sun care ingredients that makes it possible for organic sunscreen actives to be significantly reduced while maintaining key SPF measures. This step change in sun care ingredients will likely continue to spur new innovations for consumers in the area of sun care protection.

James (Vince) Gruber is the Chief Innovation Officer at Botaneco. He is located at the new Botaneco US offices and laboratories in Lambertville, New Jersey, where he oversees the technical teams at both the Calgary, Alberta, Canada, and New Jersey locations. Gruber has over 25 years of experience at various companies supplying ingredients to the personal care and cosmetic industry. He can be contacted at vgruber@botaneco.com.

Eutectic solvent for the treatment of agro-industrial low-grade oils

Adeeb Hayyan

Huge quantities of low-grade oil, such as acidic crude palm oil (ACPO) and low-grade palm oil (LGCPO), are produced as by-products of vegetable oil milling processes. Unfortunately, the inherent high acidity, or free fatty acid (FFA) content, of these by-products is a key obstacle to reusing them in other palm oil refining processes or producing biodiesel from them, because ACPO or LCGPO must first be pretreated to convert the FFA into fatty acid methyl esters (FAME).

- **Deep eutectic solvents (DESs) have realistic potential to replace numerous conventional solvents.**
- **DESs are prepared simply by mixing a hydrogen bond donor with a salt. Advantages of utilizing DESs include negligible toxicity risk, high biodegradability, high thermal stability, and low volatility compared to conventional solvents.**
- **DESs can be tailor-designed to specific applications, and thus can be applied to different types of reactions such as esterification of free fatty acid for biodiesel production.**
- **Conversion of hygroscopic organic acids such as *p*-toluenesulfonic acid monohydrate (PTSA) into DESs provides opportunity to improve the catalytic activity of strong acids.**

Consequently, the development of a catalyst can be considered to be a major contribution to pre-treatment for biodiesel processing. Currently, much of the academic focus is directed toward improving catalytic activity for biodiesel production from waste oils and fats (Canakci, 2007). Many attempts have been made to produce biodiesel from LGCPO using new types of catalysts, such as trifluoromethanesulfonic acid, benzenesulfonic acid, (1R)-(-)-camphor-10-sulfonic acid, 1-propanesulfonic acid, and chromosulfuric acid (Hayyan *et al.,* 2015). Catalyst separation and product purification are the main obstacles encountered while using homogeneous catalysts in biodiesel production. Thus, recycling of homogeneous catalysts is a primary reason for losing catalyst during various chemical reactions.

To improve the homogeneous catalysis, a new generation of catalyst known as deep eutectic solvents (DESs) for the treatment of FFA (Fig. 1.) were introduced by Hayyan *et al.*, (2013a).

DESs comprise mixtures of organic salts, such as choline chloride with a hydrogen bond donor (HBD)—an amide, organic acid, polyalcohols, amino acid, fructose, or many others (Hayyan *et al.,* 2012; Mbous *et al.*, 2017). Figure 2 shows different types of DESs with different colors.

Practically, some DESs can act as catalysts during esterification reactions. Some DESs have a sulfonic functional group, and this group is responsible for protonation and conversion of FFA to FAME (Hayyan *et al.*, 2013b).

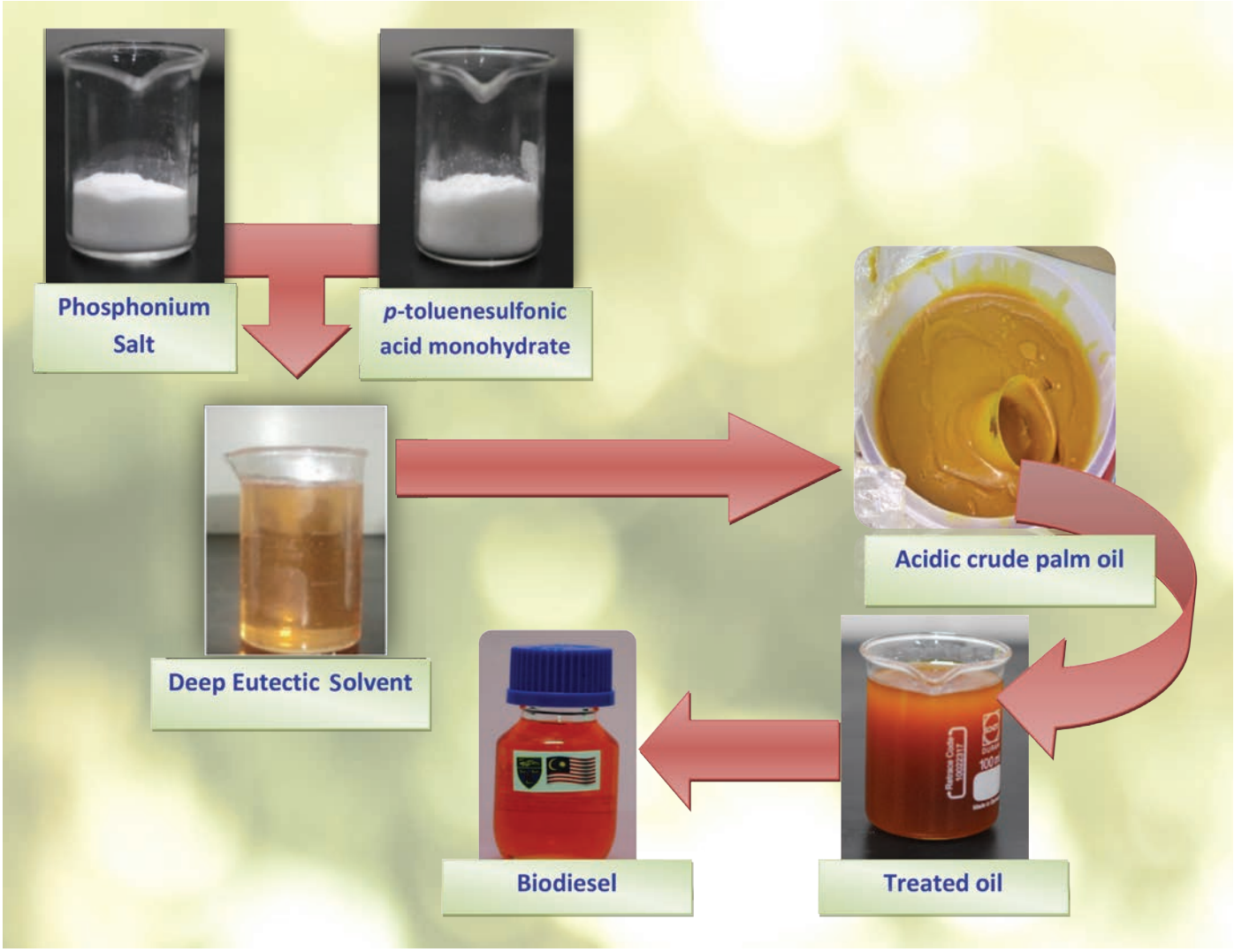


FIG. 1. Biodiesel from low-grade palm oil using DES

During the reaction, DESs are typically in homogenous phase with other reactants. The liquid state of a DES is produced through freezing point depression, which arises from hydrogen-bonding interactions between the salt's anion and the hydrogen bond donor (HBD) (Hayyan *et al.*, 2013c; 2014).

DESs are non-reactive with water. Furthermore, the toxicological profile of the components used for DESs have been determined and are available, with some DESs classified as biodegradable and environmentally friendly (Fig. 3, page 34).

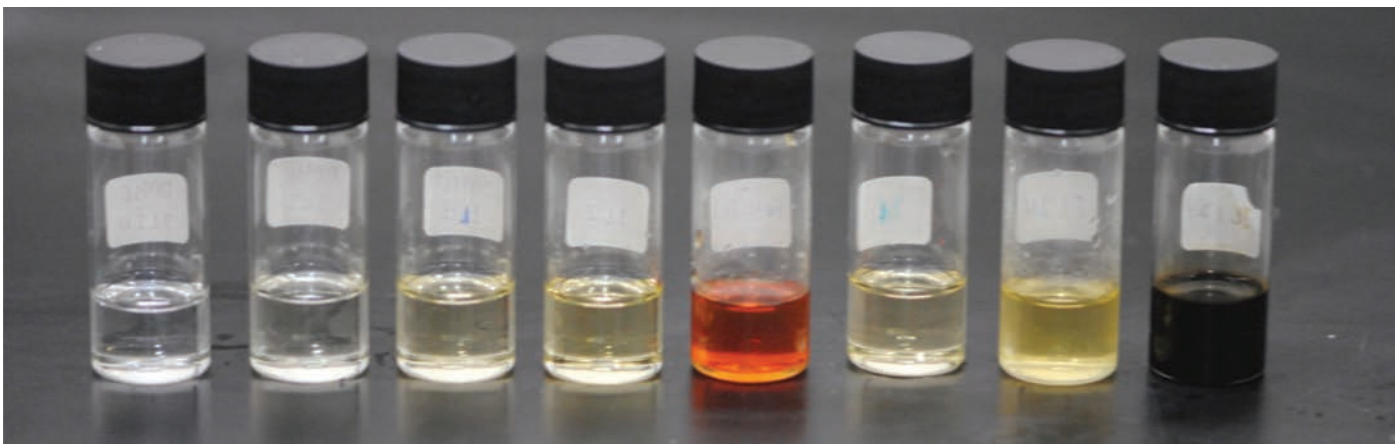
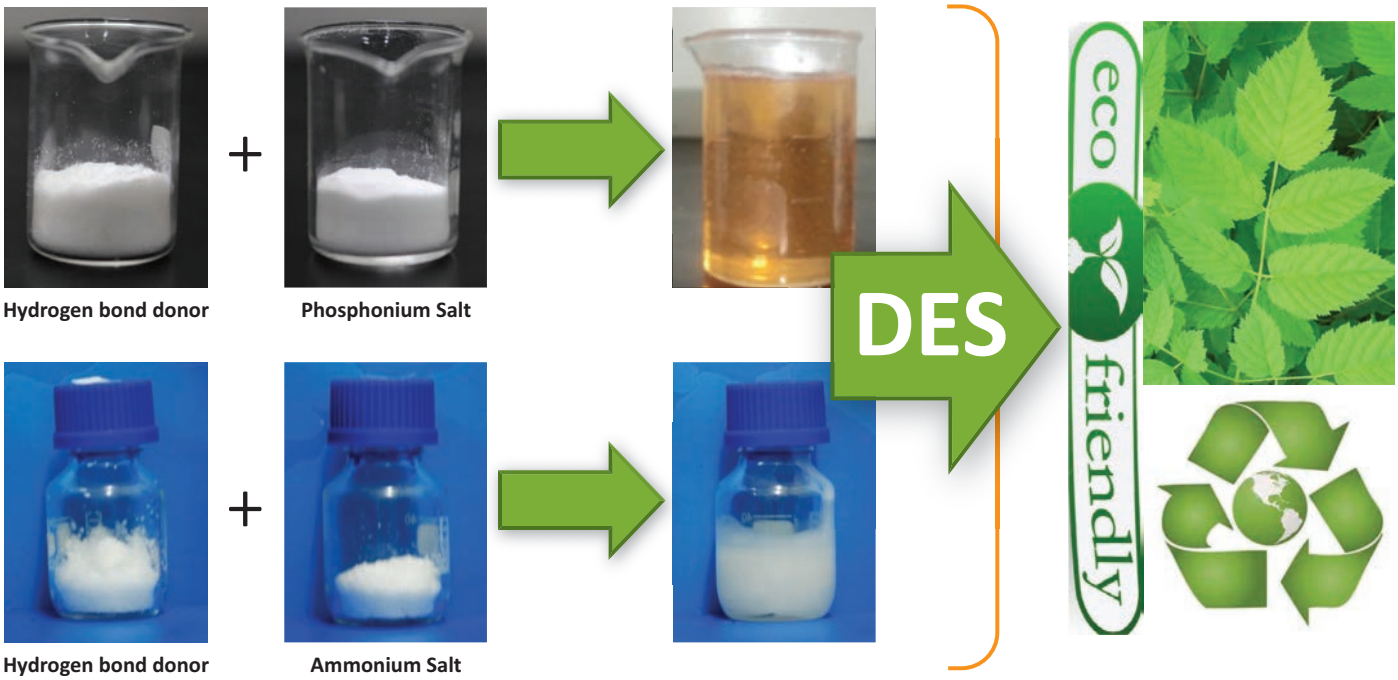


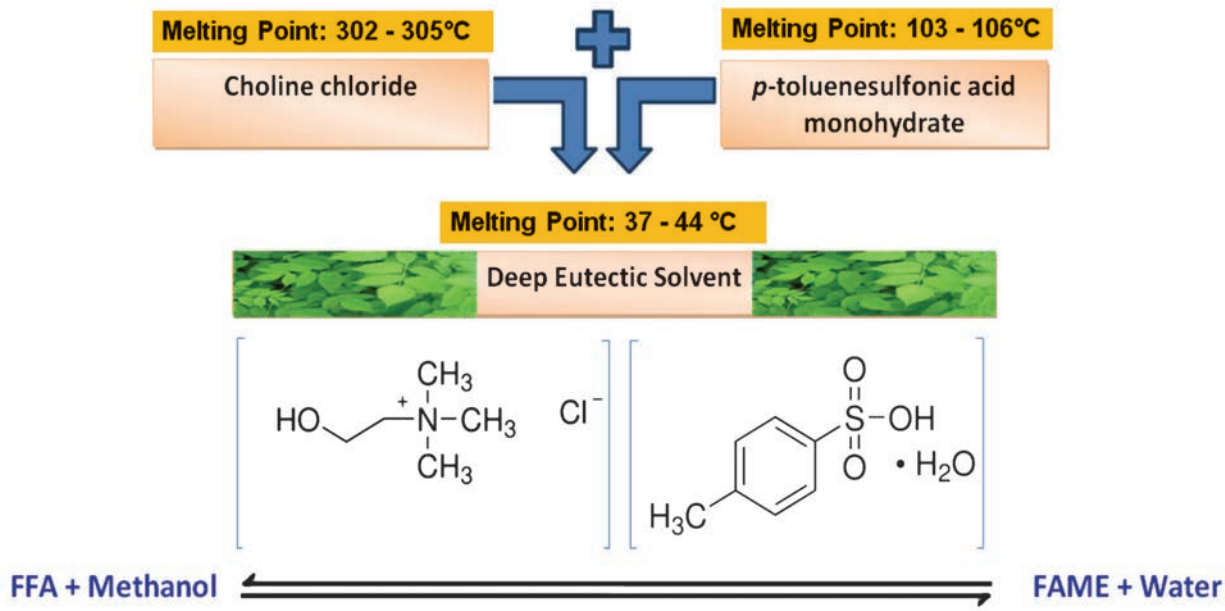
FIG. 2. Different types of DESs

FIG. 3. Ammonium- and phosphonium-based deep eutectics



Hayyan *et al*., (2013c) showed that that ammonium-based DESs exerted no toxic effect on various bacteria. On the other hand, phosphonium-based DESs showed higher toxicity compared with ammonium-based DESs (Hayyan *et al*., 2013d). However, the cytotoxicity of selected ammonium-based DESs was much higher than that of their individual components (e.g., glycerine, choline chloride), indicating that their toxic mechanism was different from their individual ingredients (Hayyan *et al*., 2013c). The toxicity and cytotoxicity of DESs varied depending on the structure of their components. These solvents have been the focus of several applications due to their desirable properties, such as high thermal stability, low volatility, low combustibility, and high conductivity (Zhang *et al.,* 2012; Smith *et al.,* 2014). DESs offer a major advantage over traditional solvents in that they can be prepared in a pure state with minimal efforts (Mbous *et al*., 2017).

FIG. 4. Melting point reduction of hydrogen bond donor and the salt Reprinted with permission from Hayyan, A., *et al*., J. Cleaner Prod. 65: 246–251, 2014.



Ionic liquids (ILs) have been used for many chemical reactions (Yue *et al*., 2011). Elsheikh *et al*., (2011) used an IL in the esterification of FFA in crude palm oil. Alternatively, cheaper, relatively green DESs were used recently in numerous applications to replace ILs and conventional solvents. DESs share many properties with ILs (Hayyan *et al*., 2012). They are biodegradable, nonflammable, and have low toxicity compared to ILs (Hayyan et al., 2013a). DESs have a low cost in comparison to ILs. For example, the cost of 100 gm of ammonium-based DES is RM 18$, while cost of some ILs may reach RM 180$.

DESs can be obtained in liquid (Fig. 2.) or jelly form (Fig. 3.), and they have much lower melting points than those of their individual constituting compounds (salt and hydrogen bond donors) as shown in Figure 4.

Operations with conventional homogenous catalysts were hampered by their hygroscopic nature, storage, and handling difficulties, which subsequently stalled their commercial application. Conversion of hygroscopic organic acids such as *p*-toluenesulfonic acid monohydrate (PTSA) into DES provides an opportunity to improve the esterification reaction.

Biodiesel production from the available industrial feedstocks will decrease the production costs for biodiesel fuel, consequently increasing the value of low-grade palm oil and opening new markets and new income sources for palm oil-producing countries such as Indonesia and Malaysia.

Adeeb Hayyan obtained his Ph.D from University of Malaya with distinction "summa cum laudae." He is currently employed as Senior Lecturer at Institute of Halal Research University of Malaya (IHRUM) and University of Malaya Centre for Ionic Liquids (UMCiL), University of Malaya Kuala Lumpur, Malaysia. Hayyan received many certificates and recognition from Elsevier and Thompson Reuters for his published papers. He received many international awards such as Young Chemical Engineer in Academia (Highly Commended) from IChemE, Manuchehr Eijadi Award from AOCS and 2015 ProSPER.Net-Scopus Young Scientist Award (2nd runner up) in Sustainable Development. His main interest is biodiesel from low grade oil, deep eutectic solvent and catalysis. He can be contacted at adeeb.hayyan@yahoo.com.

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A comparison of four common bleaching clays

Mona El-Hamidi and Ferial A. Zaher

Crude vegetable oils derive their color from the pigments that naturally occur in oilseeds, including carotenoids, chlorophylls, xanthophylls, gossypol, and gossypol derivatives. Most of these pigments are removed during alkali refining, but the chlorophylls and carotenoids that remain in the oil are removed by adsorbent bleaching—a process whereby a special affinity between the pigments and a suitable clay, or adsorbent, causes the pigments to leave the oil and adhere (adsorb) to the adsorbent. The efficiency of this process depends on the type of adsorbent used, the nature of the pigments, and other factors such as bleaching temperature and mode of bleaching. Consequently, adsorbents with a stronger affinity for carotenoids are generally more suitable for bleaching carotenoid-rich palm oil, whereas adsorbents with a greater affinity for chlorophylls are more suitable for chlorophyll-rich oils, such as olive oil.

- **The efficiency at which the bleaching process removes natural colors from vegetable oil depends on the type of adsorbent used, the nature of the pigments, and other factors such as temperature and mode of bleaching.**
- **Phenolic compounds may also be removed during bleaching, which reduces an oil's oxidative stability and shelf life. Therefore, the selection of the most suitable adsorbent should be one that maximizes bleaching efficiency while minimizing the effects of bleaching on oxidative stability.**
- **Our research group recently compared the color- and phenolic compound-removing abilities of four types of adsorbents commonly used in industrial oil bleaching.**

Phenolic compounds may also be adsorbed and removed from vegetable oils during bleaching. Such compounds are powerful antioxidants that help delay oxidation and improve odor and shelf life, so their removal can reduce the oxidative stability and shelf life of the oil. Therefore, the selection of the most suitable adsorbent should be one that maximizes bleaching efficiency while minimizing the effects of bleaching on oxidative stability.

Our research group in the Department of Fats and Oils at the National Research Centre in Egypt recently compared four types of adsorbents that are commonly used in industrial oil bleaching. Since carotenoids and chlorophyll are the two major pigments that usually remain in vegetable oils after chemical refining, we tested how efficiently each of the four clays adsorbed these two types of pigments. We also compared the effects the four clays had on the removal of desirable phenolic compounds from vegetable oils during the process of oil de-colorization.

PREPARING THE SAMPLES

The adsorbents were selected from the adsorbents most commonly used to de-colorize vegetable oils: Fulmont, Tonsil Optimum N (Tonsil N), Tonsil ACC (T. ACC), and a factory-grade bentonitic clay made in Mexico (Mexican).

Chlorophyll and carotene pigments were extracted from their natural sources and blended with two samples of a refined, bleached, and deodorized edible grade sunflower

TABLE 1. Adsorption constant and exponent constant in case of pigment adsorption on Fulmont clay

	At 30°C			At 100°C		
Pigments	Adsorption constant (k)	Exponent constant (n)	Correlation coefficient (R^2)	Adsorption constant (k)	Exponent constant (n)	Correlation coefficient (R^2)
Chlorophyll	$10^{1.0942}$ = 12.4222	0.2456	0.9819	$10^{1.1374}$ = 13.7214	0.2341	0.9614
Carotenoids	$10^{1.4447}$ = 27.8419	0.5412	0.9531	$10^{1.769}$ = 58.7489	0.8011	0.873

oil (SFO) purchased at a local market. The carotenoids were extracted from the roots of carrots (*Daucus carota*) whereas the chlorophyll was extracted from fresh leaves of the rocket plant (*Eruca sativa*) according to AOAC (2012).

Both pigment extracts were blended with the SFO to yield two oil blends: one rich in chlorophyll (A), the other rich in carotenoids (B). The concentration of pigments in the oil blends was adjusted so it would have reasonable absorbance(s) at the wave length(s) specific for each pigment. Chlorophyll absorbs at 670 nm, whereas, carotenoids absorb at 432, 455, and 480 nm. The absorbance of an oil at 670 nm and the sum of its absorbance at 432, 455, and 480 nm are thus proportional to its content of chlorophyll and carotenoids, respectively.

BLEACHING TESTS

Twenty grams of the oil containing the pigment extract (chlorophyll or carotenoids) were heated to the desired temperature, stirred with the specified load of the clay for 10 min, and then filtered. The absorbance of the filtered oil was then measured at the wavelength(s) specific for each pigment using a UV-visible spectrophotometer (UV-160 1PC, UV-visible spectrophotometer, Shimadzu, Tokyo, Japan). The procedures were conducted at 30 and 100˚C, and repeated at each temperature using different clay loads. The oil absorbance(s) after bleaching were recorded each time at the previously mentioned wave lengths.

Four samples of an edible grade SFO, 20 g each, were bleached following the pre-mentioned procedure at 100˚C using 1% of each clay (conditions resembling those usually used in industrial bleaching). The removal of carotenoids as well as chlorophyll from vegetable oils were followed up according to the reduction in the oil absorbance by bleaching at the wave length(s) at which each of these two pigments absorbs.

As previously mentioned, chlorophyll absorbs at 670 nm, whereas carotenoids absorb at 432, 455, and 480 nm. The absorbance of an oil at 670 nm and the sum of its absorbances at 432, 455, and 480 nm are thus proportional to its content of chlorophyll and carotenoids, respectively. The adsorption of pigments on bleaching clays is supposed to obey Freundlich adsorption equation, $x/m = kCn$ in which m refers to the load of the adsorbent g/100 g oil, while C refers to the concentration of the pigment after bleaching and x refers to the difference between its concentration before and after bleaching, R and C, respectively (Patterson, 1992). In this equation k is referred to as the adsorbent constant, while n is another constant called the exponent constant. According to Freundlich equation, the plotting of log (x/m) on the Y axis against log C on the X axis will yield a straight line, whose slope is equal to n and its intercept with the ordinate equals log k (Megahed, 1991). However, it

TABLE 2. Derived correlations between percentage removal of chlorophyll (y) and percentage of clay used (x) and the correlation coefficient (R^2)

Clay types	Correlation at 30°C	R^2	Correlation at 100°C	R^2
Tonsil ACC	$y = -76.567x^2 + 194.01x - 31.464$	0.999	$y = -22.003x^2 + 113.97x - 6.6061$	0.997
Mexican	$y = -46.6x^2 + 129.51x - 24.82$	0.979	$y = -63.159x^2 + 164.53x - 28.416$	0.999
Fulmont	$y = -34.12x^2 + 106.14x + 13.35$	0.999	$y = -28.27x^2 + 103.61x + 14.836$	0.999
Tonsil N	$y = -8.2073x^2 + 59.167x + 1.5364$	0.862	$y = -88.711x^2 + 184.37x - 27.473$	0.998

TABLE 3. Derived correlations between percentage removal of carotenoids (y) and percentage of clay used (x) and the correlation coefficient (R^2)

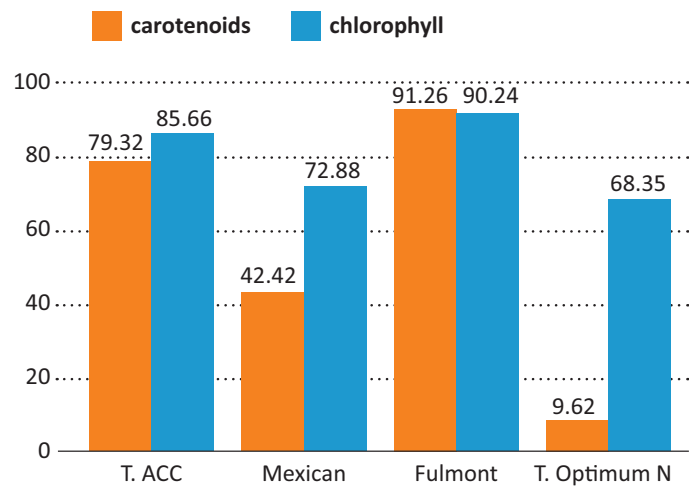
Clay types	Correlation at 30°C	R^2	Correlation at 100°C	R^2
Tonsil ACC	$y = 56.033x^2 - 43.429x + 13.566$	0.984	$y = -259.21x^2 + 417.83x - 80.356$	0.994
Mexican	$y = 3.7189x^2 - 4.7839x + 1.9718$	0.9948	$y = -10.804x^2 + 81.266x - 31.848$	0.9869
Fulmont	$y = -50.637x^2 + 134.06x + 0.0176$	0.978	$y = -60.207x^2 + 102.62x + 48.454$	0.942
Tonsil N	$y = -0.162x^2 + 2.3642x + 0.3797$	0.979	$y = 39.097x^2 - 43.984x + 14.507$	1

has been reported that adsorption of coloring matters on some types of adsorbents does not follow that equation (Gutfinger and Letan, 1978). It is clear from the results that the adsorption of chlorophyll as well as carotenoids on Fulmont clay obeys the Freundlich equation. A summary of the results is listed in Table 1. However, the results obtained in this study concerning the removal of chlorophyll and carotenoids on the other three clays used in this study were not in agreement with the Freundlich adsorption equation. This has been similarly reported with some types of clays (Gutfinger and Letan, 1978; El-Nomany *et al*., 2014).

Therefore, the efficiencies of the four clays to adsorb these two pigments from vegetable oils were studied and compared according to their percentage removal by bleaching using each clay. The correlations obtained by fitting the data in these figures which show the effect of clay load on the percentage removal of each of the two pigments are listed in Tables 2 and 3 (page 37).

These equations were used to estimate the percentage improvement in pigment removal due to the increase of the oil temperature from 30–100˚C during the bleaching process using different types of clays at different loads. It is quite clear

FIG. 1. Percentage removal of chlorophyll and carotenoids using 1% of each of the four types of clays at 100˚C



that temperature is a much more controlling variable in the case of carotenoids as compared to chlorophyll, and removal varies greatly according to the type of clay used in bleaching. For example, the increase in the temperature effects an increase in the percentage removal of carotenoids equivalent to 3,718-, 234-, 210-, and 5-fold by bleaching using 1% of Mexican clay, Tonsil N, Tonsil ACC, and Fulmont clay, respectively. However, in the case of chlorophyll, such increase in temperature effects an increase in percentage removal equivalent to 35.8-, 35.85-, 5-, and 2.6-fold only for the same clays in the same order. It appears also that the role of temperature as a controlling variable differs according to the clay load used. At lower clay loads, the effect of increasing the temperature on improving the adsorption efficiency is more pronounced in the case of Mexican clay and Tonsil ACC, while the reverse is true in the case of the other two clays (Tonsil N and Fulmont clay). In view of the pre-mentioned results, it is highly recommended to increase the temperature during the bleaching of oils rich in carotenoids, such as palm oil, especially when the clay load used is low. However, the increase in the bleaching temperature of oils rich in chlorophyll, such as olive oil, will not affect an appreciable improvement in the removal of this pigment. Hence, it is not recommended to heat such oils during the bleaching process as to reduce energy consumption and hence the running cost of the bleaching process.

The capacities of the different clays to adsorb chlorophyll as well as carotenoids at 100˚C using 1% clay (which are the conditions usually adopted for oil and soap industrial bleaching) are compared in Figure 1. Fulmont clay seems to have the greatest power to remove both types of pigments, whereby the percentage of their removal using this clay was over 90%. The adsorption power of other clays decreases in the following order: Tonsil ACC>Mexican>Tonsil N. The effect of clay type on pigment removal is more pronounced in the case of carotenoids compared to that in the case of chlorophyll. The percentage of carotenoids removal using Fulmont clay was 91.3% compared to 9.62% only using Tonsil N. Thus the latter type of clay, Tonsil N is not recommended to bleach oils rich in chlorophyll.

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PHENOLIC CONTENT ANALYSIS

The bleached oil samples, as well as the original oil sample (a total of five oil samples), were then tested for their phenolic content in two consecutive steps: 1. extraction of phenolic compounds from the five oil samples 2. quantitative determination of total phenolics using a Folin-Ciocalteu (FC) reagent.

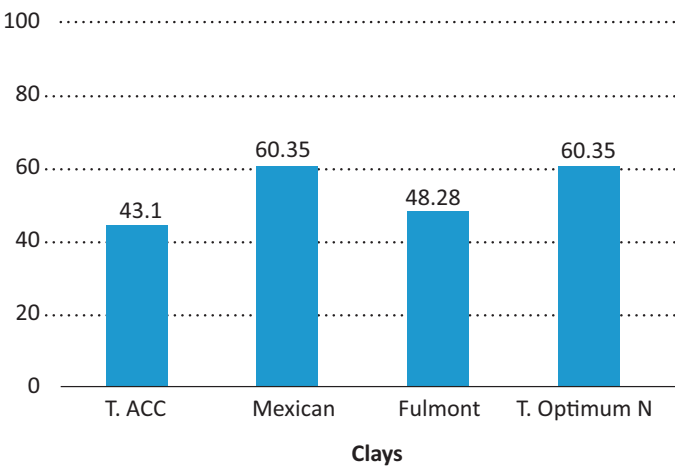
The phenolic compounds were extracted using the procedure of Tasioula-Margari and Okogeri . Briefly, 10 mL of n-hexane was added to 10 mL of each oil sample, and shaken for one minute followed by the addition of 10 mL of MeOH/H_2O mixture (70/30, v/v). The whole mixture was then thoroughly shaken in a separating funnel, and the lower layer containing the phenolic compounds was then separated. The extraction procedure was repeated twice with another 10 mL MeOH/H_2O mixture. The two extracts were collected, and the volume was made up to 25 mL with the same solvent mixture.

The method of Singh *et al*. was used to estimate the percentage of total phenolic compounds in the oil samples using Folin-Ciocalteu (FC) reagent with slight modification according to El-Hamidi and El-Shami. From each of the phenolic extracts, a suitable volume (100 µL) was taken, and the volume was brought up to 3 mL with distilled water. Two milliliters of the FC reagent that had been diluted 10-fold was then added. After 5 min, 1 mL of 7.5% sodium carbonate solution was added, and the sample was left to rest for 30 min at room temperature before spectrophotometric readings were taken. The absorbance was measured at 765 nm using a UV-visible. A blank was prepared exactly the same way with exclusion of phenolic extracts. The percentage removal of total phenolic compounds using the four different adsorbents were then estimated and compared.

THE EFFECTIVENESS OF THE FOUR CLAYS COMPARED

The four types of clays were compared for their effect on removing phenolic compounds from vegetable oils (SFO) during their bleaching (Fig. 2.) The removal of phenolic compounds from vegetable oils (SFO) was least using Tonsil ACC clay, followed by Fulmont clay. Mexican clay and Tonsil N have almost the same power to remove phenolic compounds. Hence, it is expected that oils bleached using Tonsil ACC will be the most stable against deteriorative oxidation during their use or storage. However, they are slightly darker than those bleached using Fulmont clay.

FIG. 2. Percentage removal of phenolic compounds of sunflower oil (SFO) using 1% of each of the four types of clays at 100˚C



According to the results of this study, it can be stated that carotenoids can be adsorbed on the bleaching clays more efficiently than chlorophyll. In addition, the effect of temperature on pigment removal by bleaching is much more pronounced in the case of carotenoids compared to chlorophyll. Also, it has been found that bleaching of vegetable oils using Fulmont clay can yield oils of lighter color compared to other clays, while their bleaching using Tonsil ACC clay can yield oils more stable to oxidation.

Mona El-Hamidi and Ferial A. Zaher work in the Department of Fats and Oils, National Research Centre, in Giza, Egypt.

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Rendered products in pet food: delivering protein and sustainability

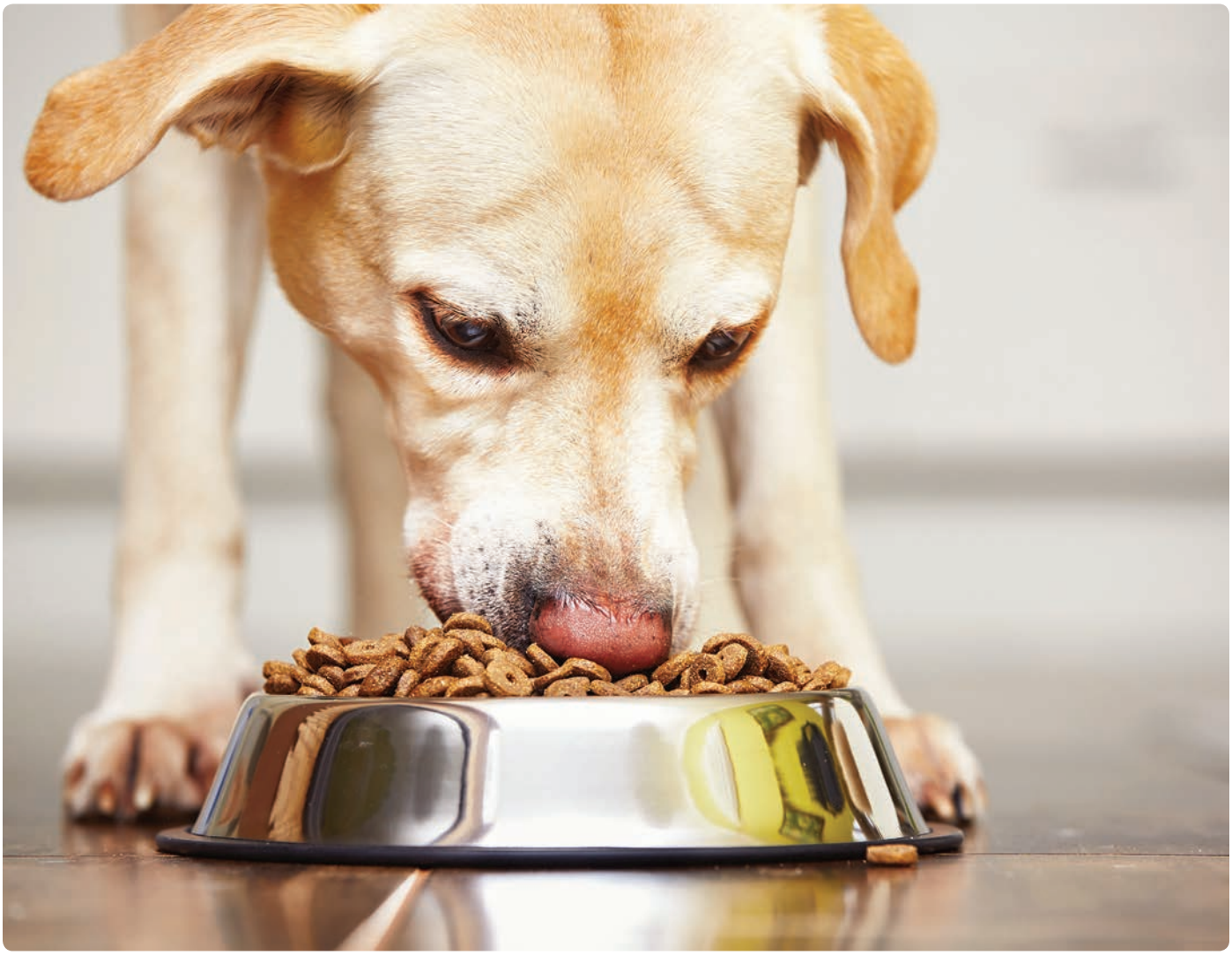
Kelly S. Swanson and Maria R.C. de Godoy

- **Nutritional sustainability is the ability to provide safe and adequate nutrition to maintain health in a population without compromising the nutritional needs of future generations.**
- **Animal-based proteins and fats typically have a larger footprint than plant-based sources, but the calculations are based on foods meant for human consumption.**
- **In addition to being safe and highly nutritious ingredients, animal proteins and fats used in pet food are secondary products of the human food system and essentially reduce the footprint of human foods.**

Americans currently have an estimated 170 million pet cats and dogs, outnumbering children by a 4-to-1 margin (APPA 2016). Many of these animals live inside homes and are important members of the family. As with humans, the nutrition, health, and lifespan of these furry companions are top priorities. The emotional tie that people have with their pets and the increasing humanization of these animals in society is reflected in the steady growth, marketing trends, and spending patterns of pet-related products and services. Although American pet ownership numbers have remained relatively stable over the past few decades, annual pet expenditures in the United States have grown from approximately $17 billion in the mid-1990s to over $60 billion today (APPA 2016).

The largest industry serving pets is the production of pet food and treats, which is nearing the $25 billion mark in the United States and is over $70 billion worldwide. Revenue growth in the United States is not due to greater volume sold but to a shift toward higher-quality products that are more expensive. Although price, marketing terminology, or pet food category do not necessarily translate to quality, products deemed to be superior by owners are often chosen. Pet food trends are increasingly following that of human food, with the terms "natural," "organic," and "fresh" being increasingly used.

In addition to the attention these particular product segments have received lately, the ingredient and nutrient profiles of pet foods are heavily scrutinized by today's consumers. Although domestic dogs are now more omnivorous in nature—they evolved eating high-protein, high-fat diets—cats are still strictly carnivores today. Therefore, protein is the nutrient class that usually attracts the most attention, with diets containing increased amounts and animal-based proteins being more popular. The terminology used on pet food labels is also important, with many owners preferring ingredients similar to those listed on human food labels.



The rendering industry plays an important role in the production of commercial pet foods, with about 30% of animal protein meals and 15% of animal fats produced in the United States making their way into such products (Informa Economics 2011). While the pet food industry provides many opportunities for sustained or increased revenue for renderers in the future, several challenges and research needs also exist and must be considered.

First, on the positive side, consumers continue to demand pet foods containing increased protein concentrations and are often willing to pay a premium for those of superior quality. Second, animal-based ingredients have a high protein quality (amino acid profile) in comparison to plant-based sources and are highly digestible if processed appropriately. Finally, rendered products are decidedly sustainable (Meeker and Meisinger 2015).

Moving forward in a world with a rapidly increasing human population and limited supply of land and water resources for food production, the use of sustainable ingredients in pet foods will be essential. For any food system to be sustainable, it must meet the needs of the present without compromising those in the future, considering environmental, economic, and social issues. Environmental factors to be looked at include global warming potential/greenhouse gas emissions (carbon footprint); land use; water use, acidification, and pollution (water footprint); and soil quality, waste management, and biodiversity of wild plants and animals.

When evaluating a food system where a standard of nutrition and health of the consumer must be maintained for it to be considered sustainable, the discussion should be taken one step further toward the concept referred to as "nutritional sustainability" (Deng and Swanson 2015). Nutritional sustainability is the ability to provide safe and adequate nutrition to maintain health in a population without compromising the nutritional needs of future generations (Swanson *et al*. 2013). Few ingredients or foods are either sustainable or unsustainable but rather are on a continuous scale of sustainability. A wide range of sustainability scores exist and depend on source (e.g., animal or plant), production strategy, global region, and more. Although animal-based proteins and fats typically have a larger footprint than plant-based sources, the calculations are based on foods meant for human consumption. Rendered products are unique in this regard as they do not compete directly with human food. In addition to being safe and highly nutritious

ingredients, animal proteins and fats are secondary products of the human food system and essentially reduce the footprint of human foods. This will continue to be an important concept for the rendering industry to embrace and promote.

Despite the positives that exist, the rendering industry faces various pressures and challenges from regulatory bodies, animal activists, and pet owners. While few take it to the extreme by demanding the use of animals and animal products be eliminated altogether, a considerable portion of the population has developed a negative connotation with the term "by-product" when it comes to pet food. Although the pet food industry was established and is still largely based on the use of secondary products of the human food system, a perception of inferiority is often attributed to animal by-products. In addition to a few pet food companies that have aggressively marketed against the use of these ingredients in the recent past, the nonstop digital media that exists in the world today has likely contributed to this viewpoint. Furthermore, anecdotal evidence and opinions found on the internet are often accepted as facts. Similar to pet food companies that use animal proteins and fats in their formulas, the rendering industry must strategically and actively promote the benefits of their ingredients.

One of the best ways to demonstrate value and promote the use of rendered products is by conducting and publishing the results of novel research studies. Considerable emphasis must be placed on areas in need of immediate solutions and opportunities to demonstrate the high quality or value of these products should not be overlooked. For instance, ingredient consistency—in terms of nutrient profile, quality, and digestibility—is one of the biggest challenges with the inclusion of animal-based proteins and fats in pet foods today, and is an area in great need of research. The identification, isolation, or testing of value-added products with specific nutrition- or health-related properties is another potential avenue of research. For example, the characterization and/or evalua-

PET FOOD SUSTAINABILITY PAPER PUBLISHED

A peer-reviewed scientific paper entitled, "Rendered ingredients significantly influence sustainability, quality, and safety of pet food," was published in the *Journal of Animal Science* in February 2015. The paper was written by David Meeker and Jessica Meisinger of the National Renderers Association and is available for download through Render's website at www. rendermagazine.com/industry. Highlights of the article are:

- Americans tend to eat only the muscle meat from each food-producing animal, so using the by-products for pet food is one way to close the sustainability loop.
- Ownership of cats and dogs is high around the world and is only expected to increase.
- Dogs and cats are carnivores and although dogs are able to exist on properly balanced meatless diets, cats are obligate carnivores and require meat products in their diet.
- Rendered ingredients are excellent sources of protein, energy, and minerals, all of which are required in pet diets.
- As countries gain more wealth, their citizens eat more meat and own pets that also require food.
- Without affordable rendered ingredients, pet food would be more expensive.
- Using human-grade food for pet diets is unsustainable for a number of reasons, one being cost.
- Using first-use ingredients in petfood (such as mined phosphorus instead of meat and bone meal or soybean products) is unsustainable for the environment for many reasons, including requiring more fertilizer, fuel, and water.
- Rendering is one of the oldest forms of recycling and an important greenhouse gas avoidance technology.
- Rendering is a highly regulated industry that practices continuous improvement.

Of note in the article is that, "Recycling products that do not compete for human food resources and would otherwise be wasted, and sparing the amount of extra ingredients and the land, water, and nutrients to produce them, is the epitome of a sustainable process and essentially describes rendering."

The rendering industry has an aggressive approach to animal food ingredient quality and safety. Nearly all rendering plants have quality and safety control systems in place via formal programs such as the North American Rendering Industry Code of Practice. A concerted effort is made to foresee product safety hazards that are likely to occur and to prevent those from happening. Testing is used to monitor and verify that rendering processes are correctly operated and managed.

tion of novel ingredients or bioactive molecules of rendering streams, which could be done using a combination of chemical, *in vitro*, and animal testing, could be of great value to the industry. These are only a few of the many new research areas that pertain to rendered products.

In summary, the pet food industry has a history of steady growth and a future full of optimism. While the passionate consumer base driving the industry creates challenges at times, its continued demand for safe, high-quality, animal-based products provides the rendering industry with many opportunities. Those that identify, develop, and market value-added streams to their business and/or consistently produce and deliver superior ingredients to pet food manufacturers will reap the rewards the industry has to offer.

Kelly S. Swanson is a professor of animal and nutrition sciences at the University of Illinois at Urbana-Champaign, USA. He can be contacted at ksswanso@illinois.edu.

Maria R.C. de Godoy is an assistant professor of animal sciences, also at the University of Illinois at Urbana-Champaign, USA. She can be reached at mgodoy@illinois.edu.

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Further reading

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Combining **modern plant breeding and enzyme technology** to obtain highly enriched **erucic acid from Crambe oil**

Natalia Volkova, Xueyuan Li, Li-Hua Zhu, and Patrick Adlercreutz

- **A newly developed transgenic Crambe line produces seed oil with 68% erucic acid compared to 53% in the wild type oil.**
- **Further enrichment of erucic acid from Crambe oil was achieved by selective enzymatic hydrolysis.**
- **The combination of modern plant breeding and enzyme technology is a promising approach for preparation of fatty acids of high purity.**

Vegetable oils constitute a renewable raw material with great potential not only for production of food and feed but also in the production of industrial materials and fuels. Transesterification of vegetable oils to methyl esters to be used as biodiesel is carried out industrially [1], and fatty acids are used as building blocks in many products. In a typical example, a fatty acid is esterified with a fatty alcohol to produce a wax ester [2], or the wax ester is formed in the alcoholysis of a vegetable oil with a fatty alcohol [3]. Wax esters can be used for various applications, such as coatings for wood [4] or as ingredients for cosmetics [5]. Furthermore, fatty acids can be used as the hydrophobic building blocks of surfactants such as carbohydrate esters [6,7]. Still another application is the use of erucic acid and its derivatives, such as erucamide, in manufacturing plastics, nylon13-13 and high temperature lubricants [8].

Sometimes the natural mixture of fatty acids from a vegetable oil can be used in the final product, but in most cases there is a need for purification of fatty acids. In the case of production of erucamide, it is important to have erucic acid with high enough purity. Modern plant breeding techniques enable us to

develop new crop varieties in a more precise and efficient way. Through genetic transformation, we have previously obtained transgenic Crambe, a dedicated industry crop species, with a significantly increased level of erucic acid in the seed oil through expressing the *LdLPAAT*, *BnFAE1* and *CaFAD2*-RNAi genes [9,10]. However, the erucic acid level still needs to be enriched further for erucamide production.

One way to enrich fatty acids from natural oils is to use fractionation, by distillation or crystallization, or to utilize the fatty acid selectivity of lipases. Lipases often accept a wide variety of carboxylic acids as substrates, and the catalytic activity depends on how well the particular acid fits in the active site of the lipase. The ideal lipase would convert the desired fatty acid(s) much slower or faster than the other fatty acids in the original mixture. Then, the lipase-catalyzed conversion reacted and un-reacted fatty acids would be separated by extraction, distillation, and so on. A particularly successful example concerns enrichment of long-chain omega-3 fatty acids [11, 12, 13], but enrichment of erucic acid [14] and gamma-linolenic acid [15] has also been reported.

Our research group recently conducted a study to see if the erucic acid content of acylglycerols from wildtype and transgenic Crambe could be further increased by selective lipase-catalyzed hydrolysis of the oils.

TABLE 1. Fatty acid composition (in mol%) of the Crambe oils used in this study (mean ± standard deviation, *n* = 3)

Fatty acids	Wild type oil	High erucic oil
C 16:0	5.1 ± 0.2	3.9 ± 0.2
C 18:0	1.7 ± 0.8	1.4 ± 0.6
C 18:1	30.0 ± 0.8	17.8 ± 0.5
C 18:2	4.8 ± 0.2	1.3 ± 0.2
C 18:3	2.0 ± 0.2	0.7 ± 0.1
C 20:1	2.7 ± 0.4	6.3 ± 0.6
C 22:1	52.8 ± 0.5	67.9 ± 0.7

SELECTIVE ENZYMATIC HYDROLYSIS

The fatty acid compositions of the wild type oil and the high erucic oil are shown in Table 1. The high erucic oil contained substantially larger amounts of erucic acid and C20:1 than the wild type oil. On the other hand, the contents of palmitic and stearic acids were lower, and those of linoleic and linolenic acid were substantially lower in the high erucic oil compared to the wild type oil.

To enrich erucic acid further, selective enzymatic hydrolysis was attempted. The method is based on the fatty acid selectivity of lipases in which a lipase would be expected to selectively hydrolyse off other fatty acids from the triacylglycerols. After separation of the acylglycerol and free fatty acid fractions (by extraction, distillation, and so on), a product further enriched in erucic acid could be obtained. Previous model studies using equimolar mixtures of erucic acids and other relevant fatty acids showed that the lipases from *Candida rugosa, Thermomyces lanuginosus*, and *Pseudomonas cepacia* express the desired fatty acid selectivity [16], although the selectivity is not quite as strong as in cases involving long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [17, 18].

TABLE 2. Maximal content of erucic acid in the acylglycerol fraction during lipase-catalyzed hydrolysis of Crambe oils

Treatment	Mol% C 22:1	
	Wild type oil	High erucic oil
Original oil	52.8	67.9
Product; *Thermomyces lanuginosus*	56	73
Product; *Pseudomonas cepacia*	62	80
Product; *Candida rugosa*	82	95

Total reaction time 40 h. Only the maximal values are shown.

TABLE 3. Fatty acid composition (in mol%) of the acylglycerol fractions after enzymatic enrichment for optimal reaction time (mentioned in parentheses)

Fatty acids	Wild type oil (25 h)	High erucic oil (11 h)
C 16:0	2	1
C 18:0	–	–
C 18:1	13	4
C 18:2	–	–
C 18:3	–	–
C 20:1	3	–
C 22:1	82	95

All three lipases catalyzed the selective hydrolysis of other fatty acids, leading to enrichment of erucic acid in the acylglycerol fraction (Table 2). Using *T. lanuginosus* lipase, only modest increases in erucic acid content were achieved for both oils. Higher erucic acid contents were achieved with *P. cepacia* lipase, and the best results were obtained with *C. rugosa* lipase.

During the initial phase of the reactions, the content of erucic acid in the acylglycerol fraction gradually increased because of hydrolysis of other fatty acids, but in the end of the reaction, the erucic acid content started to decrease again. This is because the lipase can remove even erucic acid, and this happens to an appreciable extent when erucic acid is the dominating fatty acid remaining in the acylglycerols. As in most other cases of enzymatic fatty acid enrichment, it is important to stop the reaction at a suitable reaction time. The maximum was reached in a shorter reaction time with the high erucic oil, maybe because it contained a lower amount of the fatty acids to be removed. In addition to the level of erucic acid achieved in the acylglycerol fraction, the yield of erucic acid is of importance. The erucic acid, which has been hydrolyzed from the acylglycerols, can be considered as lost (unless additional process steps are added). The recovery of erucic acid can be expressed as the ratio of erucic present in the acyl glycerol fraction and the initial amount of erucic acid in the starting material.

The recovery of erucic acid decreased with increasing reaction time, especially for the wild type oil, which decreased to very low values after 40 h reaction, indicating close to total hydrolysis of the oil. The recoveries at the maximal percentage of erucic acid in the acylglycerol fraction were about 60% for the high erucic oil and about 10% for the wild type oil. The main other fatty acid remaining in the acylglycerol fraction was oleic acid (Table 3).

HIGHER SELECTIVITY NEEDED FOR LARGE-SCALE COMMERCIALIZATION

The lipase-catalyzed enrichment caused a considerable increase in erucic acid content, but the recovery of erucic acid probably needs to be higher than that achieved in this study to be economically feasible on a large scale. A main factor which determines the outcome of the enzymatic enrichment reactions is the intrinsic specificity of the lipase for erucic acid in comparison with the specificity for the other fatty acids present in the substrate mixture. This is best quantified in terms of competitive factors [19], which are the ratios of k_{cat}/K_m of the two substrates to be compared. Competitive factors of lipases for erucic acid and other fatty acids [$(k_{cat}/K_m)_{fatty\ acid}/(k_{cat}/K_m)_{erucic\ acid}$] have been measured in the hydrolysis of fatty acid methyl esters and in the esterification of free fatty acids and were reported to be in the range 1.4–6.0 [16]. On the other hand, the corresponding competitive factors of up to over 100 have been reported for lipase-catalyzed enrichment of the long-chain omega-3 fatty acids EPA and DHA [17]. The situation is further complicated if the fatty acids are part of triacylglycerol molecules, since all acylglycerols are potential substrates and other fatty acids than the one hydrolyzed off can be expected to have an influence.

In the case of long-chain omega-3 fatty acids it has been shown that enrichment from triacylglycerols is less efficient than from simple esters [18]. There is a clear need for lipases with even higher selectivity in the enrichment of erucic acid. This could be achieved by screening among natural lipases or protein engineering of known lipases [20]. The commercial preparation of *C. rugosa* lipase, which was the best enzyme in the present study, contains isoenzymes with slightly different properties [21]. Starting with one of these isoenzymes and mutating it, Pleiss and co-workers managed to block the active site so that fatty acids longer than a critical length (C10 or C14) were strongly discriminated against [22]. This demonstrates that there are good possibilities to create new lipases with improved fatty acid selectivity and a similar methodology could be useful to prepare a lipase well suited for enrichment of erucic acid.

Plant breeding can be used to increase the content of a specific fatty acid in the seed oil to high levels, but further enrichment is often needed to achieve above 90% purity. Selective lipase-catalyzed hydrolysis was shown useful for increasing the content of erucic acid in transgenic Crambe seed

oil from 68 to 95% with a recovery of 60%. To achieve even more efficient lipase-catalyzed enrichment, lipases with even higher selectivity are needed.

Natalia Volkova and Patrick Adlercreutz are affiliated with the Department of Biotechnology at Lund University, Sweden. Xueyuan Li and Li-Hua Zhu are affiliated with the Department of Plant Breeding at the Swedish University of Agricultural Sciences.

Corresponding author Patrick Adlercreutz can be contacted at Patrick.Adlercreutz@biotek.lu.se.

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Sugar politics, then and now

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

Laura Cassiday

Similar to opposing political parties, sugar and fat are often adversaries in the great debate over which ingredient is more harmful to human health. And like all politics, nutrition politics can be dirty. In December 2016, an analysis of historical documents revealed that the sugar industry funded a favorable 1967 *New England Journal of Medicine (NEJM)* review article on carbohydrates, fat, and atherosclerosis by three prominent Harvard nutrition scientists (Kearns, C. E., *et al.*, http://dx.doi.org/10.1001/jamainternmed.2016.5394, 2016). Now, 50 years later, an industry-funded systematic review argues that dietary guidelines recommending limited sugar consumption are based on low-quality evidence (Erickson, J., *et al.*, http://dx.doi.org/10.7326/M16-2020, 2016). Some media reports have suggested that these examples reveal a "grand sugar conspiracy," whereas other sources believe that the controversy is much ado about nothing.

In the 1970s, epidemiological data from the Seven Countries Study by renowned American nutritionist Ancel Keys implicated saturated fat as the major dietary contributor to cardiovascular disease (Cassiday, L. *Inform 26*, 343–349, 377, 2015). Meanwhile, British physiologist John Yudkin published his book *Pure, White and Deadly: The Problem of Sugar* (1972), which presented epidemiological and biochemical evidence that excess sugar, and not fat, consumption is a major cause of obesity, diabetes, and heart disease. Keys' theory prevailed, and the ensuing decades witnessed a low-fat diet craze that continues today. However, recent years have seen a renewed interest in Yudkin's ideas, and increasing evidence indicates that sugar (and refined carbohydrates in general) is just as much, if not more, to blame than saturated fat for cardiovascular disease and other ailments.

Even during Yudkin's time, some individuals were accusing the sugar industry of having undue influence in the sugar–fat debate. To investigate these allegations, in 2016 Cristin Kearns and colleagues at the University of California, San Francisco, searched boxes of letters in the Harvard Medical Library for correspondence between the Sugar Research Foundation (SRF; now the Sugar Association, a sugar industry trade group based in Washington, D.C.) and D. Mark Hegsted, a professor of nutrition at the Harvard School of Public Health and co-author of the 1967 *NEJM* review on carbohydrates, fat, and atherosclerosis. Kearns discovered that in 1965, the SRF offered Hegsted and his co-authors, Harvard researchers Robert McGandy and Frederick Stare, monetary compensation for a review of papers linking sugar to metabolic diseases. In total, the SRF paid the researchers $6,500 ($48,900 in 2016 dollars) for this project.

"Our particular interest had to do with that part of nutrition in which there are claims that carbohydrates in the form of sucrose make an inordinate contribution to the metabolic condition, hitherto ascribed to aberrations called fat metabolism," wrote SRF vice president John Hickson to Hegsted on July 30, 1965. "I will be disappointed if this aspect is drowned out in a cascade of review and general interpretation."

"We are well aware of your particular interest in carbohydrate and will cover this as well as we can," replied Hegsted. The correspondence also indicates that the SRF provided relevant research papers and examined the final draft of the review, although it is unclear whether they edited or commented on drafts.

The *NEJM* review criticized studies implicating sugar in coronary heart disease, concluding that the dietary changes most likely to prevent the disease were reductions in saturated fat and cholesterol. Although the authors acknowledged a "slightly significant role for the kind and amount of dietary carbohydrate in the regulation of serum lipids," they concluded that "the practical significance of differences in dietary carbohydrate is minimal in comparison to those related to dietary fat and cholesterol" (McGandy, R. B., *et al.*, *N. Engl. J. Med.*

277, 242–247, 1967). The review did not disclose the SRF funding source; however, conflict-of-interest disclosures were not required by *NEJM* until 1984.

The Sugar Association issued a response to Kearns' article, acknowledging that the SRF "should have exercised greater transparency in all of its research activities . . . Generally speaking, it is not only unfortunate but a disservice that industry-funded research is branded as tainted . . . We question this author's continued attempts to reframe historical occurrences to conveniently align with the currently trending anti-sugar narrative, particularly when the last several decades of research have concluded that sugar does not have a unique role in heart disease."

Also in response to Kearns' article, Katherine Rich, chief executive of the New Zealand Food and Grocery Council, wrote an opinion piece for *Food Navigator* entitled, "Sugar review: Rewriting history to expose a non-existent conspiracy" (http://tinyurl.com/foodnavsugar, September 19, 2016). Rich disputes the "hysterical headlines" alleging a grand sugar industry conspiracy, and feels that "what Dr. Kearns discovered in a few musty letters doesn't warrant the reputational trashing of three Harvard nutrition professors," who are now all deceased and thus unable to respond to the allegations.

Rich notes that McGandy, Stare, and Hegsted's conclusions were in line with the thinking of most academics of the time, when the evidence for fat playing a role in heart disease was much stronger than that for sugar. Therefore, the review could have merely reflected the professors' own views. She also doubts that the researchers would risk their jobs and reputations for a combined $6,500. Because the *NEJM* article was only a literature review, and not a major piece of original research, Rich does not believe that the SRF-funded review could have been instrumental in tipping the balance in the Keys vs. Yudkin debate.

Also, Rich suggests that Kearns herself might not be completely free from conflicts of interest. Kearns is part of Sugarscience.org, a campaigning anti-sugar science group (a fact that Kearns failed to disclose). Previously a dentist, Kearns quit her job and exhausted her savings to search for evidence of industry's influence in the sugar debate. "With the correspondence exchanged between the Sugar Research Foundation and Prof. Hegsted so relatively bland and professional, Dr. Kearns seems to have worked hard to infer a darker meaning that supports the theory that the foundation's money somehow could have manipulated three Harvard nutrition professors and the outcome of the literature review," writes Rich.

Now, 50 years later, a growing awareness of the role of sugar in obesity, type 2 diabetes, and other health problems has prompted the US Department of Agriculture, the World Health Organization, and others to recommend limiting the intake of added sugars to less than 10% of total calories. Yet the sugar debate continues. A recent systematic review by Erickson and colleagues concluded that nutritional guidelines restricting sugar intake are based on low- to very-low-quality scientific evidence (http://dx.doi.org/10.7326/M16-2020, 2016). Although the authors say that their research should not be used to justify excessive sugar consumption, "At present, there seems to be no reliable evidence indicating that any of the recommended daily caloric thresholds for sugar intake are strongly associated with negative health effects," they write.

Critics are quick to point out that the research was funded by the International Life Sciences Institute, an organization that is financially supported by food and agrochemical companies including Coca-Cola, General Mills, Hershey's, and Monsanto. A scathing editorial accompanies the paper—co-authored by none other than Cristin Kearns—which suggests that journals avoid publishing studies funded by commercial interests (Schillinger, D., and Kearns, C., http://dx.doi.org/10.7326/M16-2754, 2016). Whether Erickson's paper is further evidence of a sweet conspiracy, or valid research that happens to align with the interests of the funding source, it appears that sugar politics has not changed substantially from 1967 until today.

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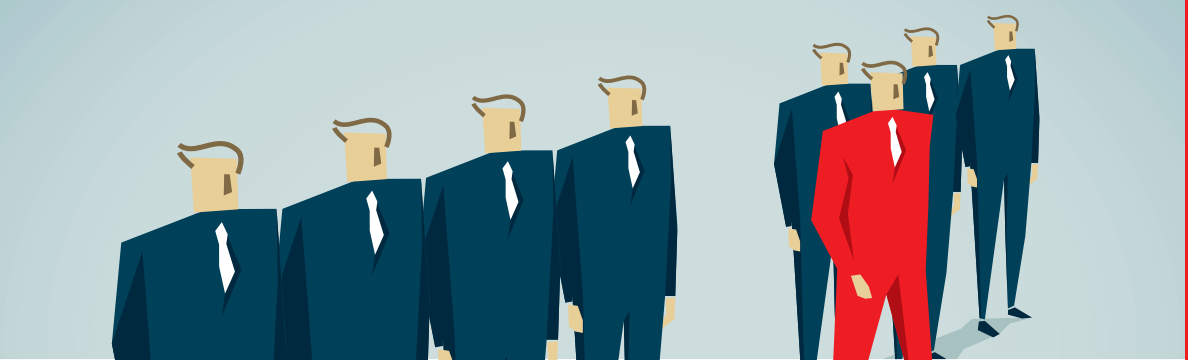
Olio is produced by Inform*'s associate editor, Laura Cassiday. She can be contacted at laura.cassiday@aocs.org.*



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Unilever US to disclose fragrance ingredients to consumers

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

Tammy Love

Unilever US has announced plans to provide consumers with information about specific fragrance ingredients, used in its personal care products.

The new disclosure initiative will cover such brands as Dove, Pond's, Nexxus, Toni & Guy, TRESemme, St Ives, Axe, and Vaseline.

Unilever says it will expand its current product ingredient lists, available through its SmartLabel website and app, to include fragrance ingredients present in a product's formulation above 0.01% (100 parts per million). Existing information provided by SmartLabel—under which consumers can view ingredient details for about 1,800 Unilever food and personal care products—lists "fragrance" but does not provide specific ingredient details.

The information expansion is aimed to be completed by the end of 2018.

The company has also announced plans to launch a webpage called What's In Our Products. This will provide additional information, including its approach to developing safe products, explanation of ingredient types and answers to common questions on SmartLabel.

Several of Unilever's US personal care products are voluntarily labelled to meet the EU's fragrance allergen labeling Regulation. This will be expanded to the full US personal care portfolio, it said.

Regarding the announcement, Unilever North America president, Kees Kruythoff, said that transparency "is fundamental to running a sustainable business.

"Through SmartLabel and What's in our Products, we are meeting the needs of our consumers who are increasingly mobile, online, and actively searching for products that are made responsibly and sustainably."

CHANGING PERSONAL CARE MARKET

The Environmental Working Group (EWG) said that Unilever's initiative "could dramatically alter the personal care and fragrance markets."

EWG president and co-founder Ken Cook called it "a game-changer" and said the NGO expects other major companies to follow suit.

"It may not happen overnight, but Unilever's watershed actions will place enormous pressure on the rest of the market to respond and make it very difficult for other companies to shield their fragrance from consumers," he added.

US Public Interest Research Group (US PIRG) called the plans "a victory for consumer product transparency." In a press release, it called on other personal care manufacturers, like Procter & Gamble and L'Oréal, to follow Unilever's lead.

But the NGO criticised its policy of only listing fragrance ingredients in a product's formulation above 0.01%, saying that it should provide full fragrance disclosure to consumers.

"For certain chemicals like endocrine-disrupting compounds, low level exposures have been associated with serious health effects," it said.

A Unilever spokesperson told *Chemical Watch* that the company had worked with fragrance suppliers to establish the threshold level. "To give some perspective, 0.01% is also the threshold level the EU set for fragrance allergen labelling for rinse-off cosmetic products," they said.

Tammy Lovell is a business reporter for Chemical Watch.

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Chile: nopal cactus as biomass for alternative energy

Leslie Kleiner

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

Sources of alternative renewable energy are of current interest worldwide. To learn more about nopal cactus as a biomass source for biogas production in Chile, I interviewed Sandra Mella (MBA, commercial engineer) and Rodrigo Wayland (food engineer), from Elqui Global Energy, Santiago, Chile.

Q: What is bioenergy, and how can nopal be used to provide it?

Bioenergy is energy produced from organic and/or industrial waste; generally this waste contains living organisms and their by-products. Bionergy is obtained from applied technology for the development of "non-conventional renewable energy" (NRCE). Biodiesel, bioethanol, and biogas are some examples of biofuels obtained from biomass and NRCE technology.

In this sense, biomass is the set of renewable organic material (plants and/or animals and/or their respective natural or artificial transformation products). The biomass can be obtained from waste or by culturing it, and can have various sources such as corn, sunflower, and nopal, to list some agricultural sources.

FIG. 1. Production at ~ 90% efficiency

TABLE 1. Equivalent power for biomass sources; (1) m3 biogas (2) 75% biogas methane equivalent power

CROP	Efficiency (L/hect-year)	Fuel	Energy (Mcal/hect-year)	Energy Kcal	Relative Energy Efficiency Base: Nopal
Nopal (Opuntia)	52.000 (1)	Biogas (1)	364,000	7.000 kcal/M3 (2)	100%
Palm	5,550	Biodiesel	51,393	9,260 Kcal / L	14%
Cocotero	4,200	Biodiesel	38,892	9.260 Kcal / L	11%
Higuerilla	2,600	Biodiesel	24,076	9.260 Kcal / L	7%
Avocado	2,460	Biodiesel	22,780	9.260 Kcal / L	6%
Jatropha	1,559	Biodiesel	14,436	9.260 Kcal / L	4%
Rape	1,100	Biodiesel	10,186	9.260 Kcal / L	3%
Soy	840	Biodiesel	7,778	9.260 Kcal / L	2%
Sugar cane	9,000	Bioethanol	45,000	5.000 Kcal /L	12%
Beet	5,000	Bioethanol	25,000	5.000 Kcal /L	7%
Yucca	4,500	Bioethanol	22,500	5.000 Kcal /L	6%
sweet sorghum	4,400	Bioethanol	22,000	5.000 Kcal /L	6%
Corn	3,200	Bioethanol	16,000	5.000 Kcal /L	4%

Nopal or prickly pear, (*Opuntia ficus indica*) is a cactus plant species that grows in warm, arid, and semi-arid regions, such as those encountered in Chile. This plant is highly resistant to extreme temperature variations, requires very little water compared to other crops, and its agricultural implementation is quite simple.

Q: Is there large-scale production of Nopal cactus bioenergy in Chile? What are its advantages over other biomass sources?

In Santiago, Chile, in the year 2000, the company Elqui Global Energy built the first biogas plant using nopal cactus as the biomass. The biogas obtained from nopal contains methane (92%), carbon dioxide (7%), hydrogen (1%), and other gases such as nitrogen; its heating capacity is 8,800 kcal/m^3.

Regarding advantages of nopal biomass for alternative energy, nopal degrades very rapidly, leading to rapid biogas production. For example, given the same volume of biogas production and usage of equipment, nopal degrades 5–10 times faster than animal manure, leading to higher productiv-

TABLE 2. Nopal biomass, wind power, and photovoltaic energy equivalent power

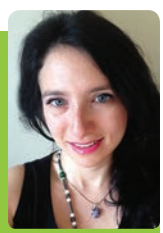
Characteristic	Energy nopal	Wind power	Photovoltaics
Availability	Continuous 24 X 365 days	Irregular depending on the time of day: 8 hours / day	Irregular depending on the time of day: 6 hours/day
Annual energy / 1 MW installed capacity	8,000 MWh	2,920 MWh	2,190 MWh
KW installed cost	US1,400 / KW	US2,200 / KW	US1,700 / KW
Payback	1 to 2 years	5-8 years	8 years
Investment / Energy produced annually	175 (US1,400.000 / 8,000 MWh)	753 (US2,200.000 / 2,920 MWh)	776 (US1,700.000 / 2,190 MWh)
Type of power generated	Electricity, biogas, thermal (hot water)	power	power
Power generation efficiency	90%	30% of installed capacity on the ground.	10–20% depending on the cost of cell
Duration equipment	20 years	20 years	20 years
environmental benefit	It generates soil and organic fertilizers. Changes the water retaining capacity of the soil. Extracts carbon dioxide from the atmosphere.	It does not emit carbon dioxide. Neutral effect on the environment.	Does not generate carbon dioxide. Neutral effect on the environment.
environmental damage	Unknown	High impact on bird migration routes, noisy energy production. Esthetically unpleasant.	Batteries and cell construction is highly polluting.
Availability of spare parts	Available in domestic market immediately.	They must be imported to Chile	They must be imported to Chile

ity. Furthermore, nopal biomass does not produce hydrogen sulfide during the process. Other advantages are its low water consumption and its ability to grow in extreme drought conditions. From a workforce perspective, the implementation of nopal as biomass for bioenergy creates local permanent jobs in traditionally rural areas. Local employees can guarantee 24h x 365 days.

Q: How does nopal biomass compare to other plant biomasses in terms of fuel and energy produced? How does it compare to other alternative energy production methods in Chile (e.g., wind power, photovoltaic)?

Such comparisons are provided in Tables 1 and 2.

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.



AOCS Journals

SPECIAL SESSION AT THE 2017 AOCS ANNUAL MEETING

On May 2nd, 2017, a special 20th anniversary session celebrating the *Journal of Surfactants and Detergents* will be held at the AOCS Annual Meeting and Industry Showcases in Orlando, Florida, USA. The session, ***Journal of Surfactants and Detergents*—20th Volume Celebration Honoring Milton Rosen**, will present individual reflections on Dr. Rosen's contributions to our community.

Speakers were invited based on their connections to Dr. Rosen and include former students, past winners of the Samuel Rosen Memorial Award, and his co-authors on publications. The response was overwhelming, and all the speaker slots filled up quickly. This is a testament to Milton Rosen as well as to the ***Journal of Surfactants and Detergents.***

The program promises to be robust and should appeal to a wide audience, as a broad range of topics related to surfactants and surfactants applications will be covered (see listing).

20th ANNIVERSARY JOURNAL OF SURFACTANTS AND DETERGENTS SINCE 1998

A quick re-cap of Milton Rosen's achievements: about 70 years working on surfactants research; over 150 patents/publications; AOCS Fellow (1999); long standing member of the advisory board for the ***Journal of Surfactants and Detergents***; 2003 winner of the AOCS-Supelco/Nicholas Pelick Award; author of several books including **Surfactants and Interfacial Phenomenon.**

Please join us at this special session!

JSD 20TH VOLUME CELEBRATION PROGRAM

S&D 3: *Journal of Surfactants and Detergents*—20th Volume Celebration Honoring Milton Rosen
Chairs: Dennis Murphy, Stepan Co., USA; and Arun Ramchandran, University of Toronto, Canada

1:55 pm	Introduction
2:00 pm	**Gemini surfactants based on Linear Alkylbenzene Sulfonate for use in Liquid Laundry Detergents.** George A. Smith, *Huntsman Corporation, USA*
2:20 pm	**Silicone Surfactants in Oil Based Systems.** Tony O'Lenick, *Siltech LLC, USA*
2:40 pm	**Synergism and Interaction of Surfactants in Enhancing Performance in Personal Care and Industrial Formulations.** Manilal Dahanayake, *Surfactant Solution Experts. LLC, USA*
3:00 pm	**Surfactant Mixtures: Synergism in Solubilization, Microemulsions, and Detergency.** David A. Sabatini, *University of Oklahoma, USA*
3:20 pm	**Surfactant-Polymer Interactions.** Yun-Peng Zhu, *Lubrizol Advanced Materials, Inc., USA*
3:40 pm	**Improve Low Tension Formulation Robustness in Enhanced Oil Recovery with Properly Optimized Surfactant Mixture.** Jean-Louis Salager and Ana Forgiarini, *Universidad de Los Andes, Venezuela*
4:00 pm	**Accounting for Ion Specific Effects in the Hydrophilic/Lipophilic Difference (HLD) Equation.** Brock A. Trotter, Mohannad Kadhum, Ben Shiau, and Jeffrey Harwell, *University of Oklahoma, USA; University of Oklahoma, University of Oklahoma, USA*
4:20 pm	**Use of High Throughput Technologies to Accelerate Formulation Development.** Christopher J. Tucker, Michael Tate, and John Ell, *The Dow Chemical Company, USA*
4:40 pm	**Samuel Rosen, Milton Rosen, and Visions of a Future Honoring a Legacy.** Charles Hammond, *Flotek Chemistry, USA*

PATENTS

Heavy crude oil viscosity reducer

Bello, C., Oil & Gas Tech Enterprises C.V., US9453157, September 27, 2016

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

Dielectric heat-transfer fluid

Knowlton, *et al.*, E.I. du Pont de Nemours and Co., US9455066, September 27, 2018

Provided is a use of a vegetable oil high in monounsaturates as dielectric and heat-transfer fluid in a device for the generation, storage, conversion, and/or distribution of electrical energy.

pH-adjusted soy protein isolate and uses

Green, B.E., *et al.*, Burcon Nutrascience (MB) Corp., US9456621, October 4, 2016

pH-adjusted soy protein products, particularly isolates, that have a natural pH of about 6 and have a non-beany flavor are provided by the processing of soy protein product which is completely soluble in aqueous media at a pH of less than about 4.4 and heat stable in this pH range or a concentrated soy protein solution produced in the preparation of such soy protein product.

Pasteurization process for microbial cells and microbial oil

Schaap, A., *et al.*, DSM IP Assets B.V., US9457108, October 4, 2016

A pasteurization protocol for pasteurizing microbial cells is disclosed. The protocol has a heating stage, a plateau stage at which the cells are held at a (maximum and) constant temperature, and a cooling stage. The heating and cooling stages are rapid, the temperature of the cells passing through 40–80˚C in no more than 30 min. in the heating stage. The heating rate is at least 0.5˚C /minute and during cooling is at least –0.5˚C/minute. The plateau maximum temperature is from 70–85˚C By plotting the pasteurization protocol on a time (t, min.) versus temperature (T, degrees centigrade), one obtains a trapezium having an area less than 13,000˚C min. This results in a smaller energy input (so a reduction in costs) and a better quality oil having a peroxide value (POV) of less than 1.5 and an anisidine value (AnV) of less than 1.0.

Algal oil-based bio-lubricants

Vijayendran, B., *et al.*, T2e Energy Holdings, LLC, US9458407, October 4, 2016

A method for manufacturing an algal oil based bio-lubricant includes selecting a base algae strain with a fatty acid profile that includes oleic acid, introducing the base algae strain to a flue gas recycling system, introducing a lipid trigger to the flue gas recycling system to enhance the lipid production efficiency of the algae, harvesting the algae, extracting an algal oil from the algae that is more than 40 percent oleic acid, and converting the algal oil into bio-lubricant using chemical modification and/or the incorporation of stabilizing additives.

Lipid-based wax compositions substantially free of fat bloom and methods of making

Murphy, T.A., *et al.*, Cargill, Inc., US9458411, October 4, 2016

Lipid-based wax compositions and their methods of making are provided for compositions substantially free of fat bloom. The compositions comprise 0.1–10% by weight triacylglycerides, 30–95% by weight monoacylglycerides and diacylglycerides combined, and 0.1–65% by weight fatty acids. The methods comprise blending the monoacylglycerides, diacylglycerides, triacylglycerides, and fatty acids by heating the lipid-based wax composition at a sufficiently high temperature to destroy substantially all crystal structure within the lipid-based wax composition. The methods further comprise pouring the lipid-based wax composition into a mold or a container having a surface and a core, wherein the pouring is conducted at a temperature at least 15°C greater than the congeal point of the lipid-based wax composition. The methods further comprise cooling the lipid-based wax composition under conditions sufficient to cool the core to at least 5°C below the congeal point of the lipid-based wax composition in 30–90 min.

Production of partially refined waste glycerol

Ko, M.K., *et al.*, REG Life Sciences, LLC, US9469586, October 18, 2016

The disclosure relates to a novel glycerol purification process that produces partially refined waste glycerol for a variety of industrial applications. The disclosure encompasses a salt-containing partially refined glycerol composition that is suitable as a fermentation grade glycerol.

Methods for die casting metals using phase separable fluids

Burke, J.M., *et al.*, Houghton Technical Corp., US9475116, October 25, 2016

Processes for die casting metals are provided, which are more energy efficient and less expensive than the current metal die casting processes. The die casting processes discussed herein utilize a water-soluble die release fluid and hydraulic equipment which uses

water-insoluble hydraulic fluid. By doing so, the hydraulic fluid and die release fluid present in the waste collected from the die casting process can be collected via separation techniques. One or both of the collected hydraulic fluid and die release fluid may then be re-used in the same die casting process, another die casting process, or other processes altogether.

Lipid compositions with high DHA content

Hallaraker, H., *et al.*, Arctic Nutrition AS, US9458409, October 4, 2016

The invention provides lipid compositions comprising phospholipids having a high docosahexaenoic acid (DHA) content, which compositions are preferably extracted from natural sources. The lipid compositions are excellent sources of highly bioavailable DHA and they can be used in oral delivery vehicles, dietary supplements, functional foods, and the like.

Process for separating polyunsaturated fatty acids from long-chain unsaturated or less saturated fatty acids

Haraldsson, G.G., *et al.*, Epax AS, US9476008, October 25, 2016

A process for separating polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from less saturated long chain fatty acids (LCFAs) in a lipid composition, wherein said PUFAs and LCFAs are present as (i) triglycerides, or (ii) free fatty acids or monoalkyl esters, by exchange of at least a portion of LCFAs with short and/or medium chain fatty acids (MCFAs). The process can suitably be employed on marine derived oil, marine oil 2 derived oil products and other sources of PUFAs, including PUFA-rich singe cell oils (SCOs), and oils from genetically modified organisms with a modified lipid metabolism. The inventive process is based on novel use of lipases and distillation techniques, selectively chemically modifying species in the substrate material such that the desired species and chemically similar species become sufficiently dissimilar to be separable. Thus PUFA can be effectively enriched from material such as 30 herring oil with low PUFA content and high content of equal length monounsaturated fatty acids such as 20:1 and 22:1 fatty acids.

Acidic methanol stripping process that reduces sulfur content of biodiesel from waste greases

Cairncross, R.A., *et al.*, Drexel University, US9476009, October 25, 2016

The present invention provides a method of producing fatty acid alkyl esters from a lipid, comprising steps of introducing a gas comprising vapor of an alcohol selected from methanol, ethanol, 1-propanol, iso-propanol, and butanols, into the lipid in a form of bubbles to enable the bubbles to pass through the lipid and be discharged from the lipid. The product may then be subjected to a transesterification process catalyzed by a base catalyst. The present invention is robust with low quality feedstocks thus significantly reduce production cost for biodiesel.

Soybean seed and oil compositions and methods of making same

Wagner, N.W., Monsanto Technology LLC, US9480271, November 1, 2016

Soybean oil compositions with unique fatty acid profiles are disclosed. These oils can be derived by the suppression of endogenous soybean FAD2 and FAD3 genes and the expression of a stearoyl acyl ACP thioesterase. Soybean plants and seeds comprising these oils are also disclosed.

Totally randomized trans-fat-free butters

O'Lenick, T.G., *et al.*, Surfatech Corp., US9481632, November 1, 2016

The present invention discloses a series of non-hydrogenated butters made by a process referred to as totally randomized triglycerides. The butters are made using a very specific range of very specific types and ratios of fatty acids to glycerin to provide the highly desirable cosmetic butters meeting the requirements 1–5 above.

Method for processing a vegetable fat composition

Miller, R.L., AAK AB PBL, US9499768, November 22, 2016

The invention relates to a method for processing a vegetable fat composition (VFC), the method comprising the steps of: providing the vegetable fat composition (VFC); in a first neutralization step (FNS) separating free fatty acids from the vegetable fat composition (VFC) thereby obtaining a neutralized vegetable fat composition (NVF); in a separation step (FRA) separating the neutralized vegetable fat composition (NVF) into a first fraction (FF) rich in StOO and/or OOO and a second fraction (SF) rich in StOSt (where St=stearic acid and O=oleic acid); in a second neutralization step (SNS) adding to the first fraction (FF) a second base (SB) thereby obtaining a neutralized first fraction (NFF); feeding the neutralized first fraction (NFF) and a stearic acid source (SAS) into an enzymatic transesterification step (ETE) transesterifying the neutralized first fraction (NFF) by using enzymes with 1,3-specific transesterification activity thereby obtaining a transesterified first fraction (TFF).

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

Whole milk increases intestinal *ANGPTL4* expression and excretion of fatty acids through feces and urine

Nielsen, S.D., *et al.*, *J. Agric. Food Chem. 65*: 281–290, 2017, http://dx.doi.org/10.1021/acs.jafc.6b04135.

The angiopoietin-like 4 (ANGPLT4) protein is involved in lipid metabolism and is known to inhibit lipoprotein lipase in the bloodstream. We investigated the effect of milk on intestinal ANGPTL4 and the metabolic profile of growing pigs and the effect of free fatty acids (FFAs) on ANGPTL4 in *ex vivo* and *in vitro* assays. Feeding pigs whole milk increased intestinal *ANGPTL4* mRNA and increased fecal excretion of long-chain FFA compared to the control group fed soybean oil (n = 9). Furthermore, FFAs (C4–C8) induced ANGPTL4 gene expression in porcine intestinal tissue mounted in Ussing chambers and ANGPTL4 protein secretion to both the apical and basolateral sides of intestinal Caco-2 cells on permeable membranes. Altogether, these results support an ANGPTL4-induced secretion of fecal FFAs. Urinary levels of FFAs (C4–C12), 3-hydroxyadipic acid, and suberic acid were also increased by milk consumption, indicating higher energy expenditure compared to the control group.

Green extraction technologies for high-value metabolites from algae: a review

Esquivel-Hernández, D.A., *et al.*, *Biofuels, Bioprod., Bioref. 11*: 215–231, 2017, http://dx.doi.org/10.1002/bbb.1735.

Cultivation of algae (micro and macro) can be used to produce several high-value metabolites to supply industries as cosmetics, additives, and pigments, among others. Those metabolites can have physiological and nutritional benefits for human and animal health. However, the availability of high-value metabolites from algae is still unaffordable due to traditional extraction techniques and their requirements of energy and use of pollutant solvents. Recently, green extraction technologies for the extraction of high-value metabolites have become more desirable due to their sustainability and environmental benefits. However, the information about green extraction metabolites from algae is limited. Therefore, this review highlights the main green extraction technologies—supercritical fluid extraction (SFE), microwave assisted extraction (MAE), and pressurized liquid extraction (PLE)—and their optimal parameters for the extraction of high-value metabolites from algae. First, general information is given regarding high-value metabolites from algae. Then, the review summarizes the principles, processes, advantages, and disadvantages of each technology. Finally, it presents recommendations and concluding remarks to select the best extraction technology.

Fatty acid intake and its dietary sources in relation with markers of type 2 diabetes risk: the NEO study

Wanders, A.J., *et al.*, *Eur. J. Clin. Nutr. 71*: 245–251, 2017, http://dx.doi.org/10.1038/ejcn.2016.204.

This study examined the relations between intakes of total, saturated, mono-unsaturated, poly-unsaturated, and trans fatty acids (SFA, MUFA, PUFA, and TFA), and their dietary sources (dairy, meat, and plant) with markers of type 2 diabetes risk. The cross-sectional analysis of baseline data of 5,675 non-diabetic, middle-aged participants of the Netherlands Epidemiology of Obesity (NEO) study looked at associations between habitual dietary intake and fasting and postprandial blood glucose and insulin. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), HOMA of β-cell function (HOMA-B), and Disposition Index were assessed through multivariable linear regression models with adjustments for demographic, lifestyle, and dietary factors. Mean (s.d.) intakes in percent of energy (En%) were 34.4 (5.8) for total fatty acids, 12.4 (2.9) for SFA, 12.2 (2.4) for MUFA, 6.9 (1.9) for PUFA, and 0.6 (0.2) for TFA. Compared with carbohydrates, only SFA was weakly inversely associated with fasting insulin, HOMA-IR and HOMA-B. When stratified by dietary source, all fatty acids from meat were positively associated with fasting insulin: total fatty acids$_{meat}$ (per 5 En%: 10.0%; 95% confidence interval: 4.0, 16.3), SFA$_{meat}$ (per 1 En%: 3.7%; 0.4, 7.2), MUFA$_{meat}$ (per 1 En%: 5.0%; 2.0, 8.1), PUFA$_{meat}$ (per 1 En%: 17.3%; 6.0, 29.7) and TFA$_{meat}$ (per 0.1 En%: 10.5%; 3.2, 18.3). Similarly, all fatty acids from meat were positively associated with HOMA-IR and HOMA-B and inversely with Disposition Index. The results suggest that the relations between fatty acid intakes and markers of type 2 diabetes risk may depend on the dietary sources of the fatty acids. More epidemiological studies on diet and cardiometabolic disease are needed, addressing possible interactions between nutrients and their dietary sources.

Lifelong imbalanced LA/ALA intake impairs emotional and cognitive behavior via changes in brain endocannabinoid system

Zamberletti, E., *et al.*, *J. Lipid Res. 58*: 301–316, 2017, http://dx.doi.org/10.1194/jlr.M068387.

Imbalanced dietary n-3 and n-6 PUFA content has been associated with a number of neurological conditions. Endocannabinoids are n-6 PUFA derivatives, whose brain concentrations are sensitive to modifications of fatty acid composition of the diet and play a central role in the regulation of mood and cog-

nition. As such, the endocannabinoid system appears to be an ideal candidate for mediating the effects of dietary fatty acids on mood and cognition. Lifelong administration of isocaloric α-linolenic acid (ALA)-deficient and -enriched diets induced short-term memory deficits, whereas only dietary ALA enrichment altered emotional reactivity in adult male rats compared with animals fed a standard diet that was balanced in ALA/linoleic acid (LA) ratio. In the prefrontal cortex, both diets reduced 2-AG levels and increased MAG lipase expression, whereas only the enriched diet reduced AEA levels, simultaneously increasing FAAH expression. In the hippocampus, an ALA-enriched diet decreased AEA content and NAPE-PLD expression, and reduced 2-AG content while increasing MAG lipase expression. These findings highlight the importance of a diet balanced in fatty acid content for normal brain functions and to support a link between dietary ALA, the brain endocannabinoid system, and behavior, which indicates that dietary ALA intake is a sufficient condition for altering the endocannabinoid system in brain regions modulating mood and cognition.

Dietary n-6:n-3 fatty acid ratios alter rumen fermentation parameters and microbial populations in goats

Ebrahimi, M., *et al.*, *J. Agric. Food Chem 65*: 737–744, 2017, http://dx.doi.org/10.1021/acs.jafc.6b04704.

Revealing the ruminal fermentation patterns and microbial populations as affected by dietary n-6:n-3 PUFA ratio would be useful for further clarifying the role of the rumen in the lipid metabolism of ruminants. The objective of the present study was to investigate the effects of dietary n-6:n-3 PUFA ratios on fermentation characteristics, fatty acid (FA) profiles, and microbial populations in the rumen of goats. A total of twenty-one goats were randomly assigned to three dietary treatments with different n-6:n-3 PUFA ratios of 2.27:1 (low ratio, LR), 5.01:1 (medium ratio, MR), and 10.38:1 (high ratio, HR). After 100 days of feeding, all goats were slaughtered. Dietary n-6:n-3 PUFA ratios had no effect ($P > 0.05$) on rumen pH and NH_3N concentration. Goats fed HR diet had lower ($P < 0.05$) propionate and total volatile fatty acids and higher ($P < 0.05$) butyrate compared with those fed the MR and LR diets. The proportion of C18:0 decreased ($P < 0.05$) as dietary n-6:n-3 PUFA ratios increased. The proportions of C18:1 *trans*-11, C18:2n-6, *cis*-9 *trans*-11 CLA, and C20:4n-6 were greater in the HR goats compared with the MR and LR goats. Lowering dietary n-6:n-3 PUFA ratios enhanced ($P < 0.05$) the proportion of C18:3n-3 and total n-3 PUFA in the rumen fluid of goats. The populations of *R. albus and R. flavefaciens* decreased ($P < 0.05$) as the n-6:n-3 PUFA ratios increased in diet. Diet had no effect ($P > 0.05$) on the ruminal populations of *F. succinogenes*, total bacteria, methanogens, total protozoa, *Entiodinium*, and *Holotrich*. The population of *B. fibrisolvens* was lower ($P < 0.05$) in the LR goats compared with the MR and HR goats. It was concluded that HR would increase the concentration of *cis*-9 *trans*-11 CLA and C18:1 *trans*-11 in the rumen. However, LR whould decrease the *B. fibrisolvens* population, which is involved in the BH process in the rumen. Further research is needed to evaluate the potential role and contribution of rumen microbiome in the metabolism of FA in the rumen.

Production of conjugated linoleic acid-rich cottonseed oil by supported Ru catalyzed isomerization

Liu, S., *et al.*, *Ind. Crop. Prod. 97*: 10–20, 2017, http://dx.doi.org/10.1016/j.indcrop.2016.12.004.

Supported metal (e.g. Ruthenium (Ru)) is capable to catalyze the isomerization of polyunsaturated fatty acids in plant oils into conjugated fatty acids. Conjugated linoleic acids (CLAs) produced from plant oils have not only been associated with diverse health and physiological effects, but also been interesting renewable compounds in producing industrial products such as paints, glues, and polymers, due to the presence of very reactive conjugated double bonds. To explore the industrial potential of heterogeneous supported metal catalyst in converting food-use limited cottonseed oil (CSO) into conjugated linoleic acid (CLA)-rich plant oil, Ru/C (J.M.), Ru/C (Sigma), Ru/Al_2O_3 and Ru black are examined for their catalytic efficiency/selectivity in decane and solvent-free systems. X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) are employed for their surface chemical composition and morphology analyses. Deconvolution of Ru 3p and 3d spectra are executed by Gaussian and Lorentzian fitting; and a qualitative correlation between oxidation states of Ru and catalytic activity/selectivity of Ru catalysts are delineated. In 24 h in solvent-free system Ru black is capable to achieve 51.3% conversion of linoleic acid (LA) in CSO with excellent selectivity (83.7%) towards CLA formation (CLA-rich CSO can be easily obtained by

simple filtration); conversion of LA doesnot show any Sn-2/Sn-1,3 region-preference; however, the reaction activity/selectivity is pronouncedly correlated to the content of Ru (IV) in catalysts.

Hydroesterification of crambe oil (*Crambe abyssinica* H.) under pressurized conditions

de Mello, B.T., *et al.*, *Ind. Crop..Prod*. *97*: 110–119, 2017, http://dx.doi.org/10.1016/j.indcrop.2016.12.014.

In this study the continuous production of esters from the hydroesterification (hydrolysis followed by esterification in two-step) of crambe oil was performed under pressurized conditions without a catalyst. For this purpose the effects of experimental variables were evaluated aiming to maximize the formation of free fatty acids (FFA) in the hydrolysis and the ester content in the esterification, keeping the pressure fixed at 15 MPa. Transesterification was also performed for comparative purposes. The results indicate that the combination of high temperature and the addition of a cosolvent (n-hexane), for a low oil to water mass ratio, favors the FFA formation. The kinetics reaction is fast and optimal conditions for the hydrolysis were identified as: temperature of 320 °C, water to oil mass ratio of 1:1, 75% of cosolvent and reaction time of 14 min. In the esterification step, increasing the temperature and molar ratio has a considerable effect and favors the FFA conversion and esters content for a residence time of ≤20 min, after which the thermodynamic equilibrium of the reaction was reached. The hydroesterification provided a higher esters content (~98%) compared to transesterification (~89%).

Analysis of sorghum wax and carnauba wax by reversed phase liquid chromatography mass spectrometry

Harron, A.F., *et al.*, *Ind. Crop. Prod*. *98*: 116–129, 2017, http://dx.doi.org/10.1016/j.indcrop.2016.09.015.

Sorghum is a genus of plant in the grass family, which is used for both grain and forage production throughout the world. In the United States, sorghum grain is predominantly used as livestock feed and in ethanol production. In recent years however, sorghum grain has been investigated for other industrial applications, including gluten-free food sources for the US food market, and waxes. The United States is the world's largest producer of grain sorghum, which is grown in the arid regions of the southern Great Plains, Arizona and California. Carnauba wax is used in a variety of products; including cosmetics, industrial polishes, food products, and paper products. The United States has no domestic source of carnauba wax, and imports 100% of its carnauba wax supply. Sorghum wax has demonstrated similar physical properties to carnauba wax, and could potentially be a viable substitute for carnauba wax. In this paper, we present the first successful reversed phase HPLC method, via a C30 column, for the analysis and characterization of waxes, without the need for specialized columns or sample derivation. Sorghum wax is composed of a heterogeneous mixture of compounds, dominated by C28 and C30 saturated and unsaturated species, while carnauba is more homogeneous in nature, and composed primarily of C56-C60 saturated wax esters.

Characterization of leaf cuticular waxes and cutin monomers of *Camelina sativa* and closely-related *Camelina* species

P. Tomasi, *et al.*, *Ind. Crop. Prod*. *98*: 130–138, 2017, http://dx.doi.org/10.1016/j.indcrop.2017.01.030.

Camelina sativa is an old world crop newly introduced to the semi-arid regions of the Southwestern United States. Recently, *Camelina* gained attention as a biofuel feedstock crop due to its relatively high oil content, polyunsaturated fatty acids, very short growing season with fairly good adaption to marginal lands, and low input agricultural systems. To expand *Camelina* growing zones into more arid regions, it is important to develop new drought resistant cultivars that can grow under water-limited conditions. Plants having cuticles with low permeability to water can possess elevated dehydration avoidance and improved drought tolerance. To extend our understating of cuticle chemical composition among *Camelina* species, leaf wax and cutin monomers in seventeen accessions representing four *Camelina* species were analyzed. *Camelina* exhibited a wide range of wax and cutin contents. The primary alcohols and alkanes were the predominant classes of leaf wax, followed in abundance by wax esters, fatty acids, aldehydes, alkylguaiacols, methylalkylresorcinols, α-amyrin and ß-sitosterol. Among primary alcohols, the dominant constituents were the C_{24}, C_{26} and C_{28} homologues, while the C_{31} homologue was the most abundant alkane among all *Camelina* accessions. Cutin monomers included monohydroxy monobasic acids, phenolics, monobasic acids, monohydroxy epoxymonobasic acids, and dibasic acids. Among the cutin monomers examined, the $C_{16:0}$ diOH acid showed extensive variation among *Camelina* species.

Photosynthetic CO_2 conversion to fatty acid ethyl esters (FAEEs) using engineered cyanobacteria

Lee, H.J., *et al.*, *J. Agric. Food Chem*. *65*: 1087–1092, 2017, http://dx.doi.org/10.1021/acs.jafc.7b00002.

Metabolic engineering of cyanobacteria has received attention as a sustainable strategy to convert carbon dioxide to fatty acid-derived chemicals that are widely used in the food and chemical industries. Herein, *Synechococcus elongatus* PCC 7942, a model cyanobacterium, was engineered for the first time to produce fatty acid ethyl esters (FAEEs) from CO_2. Due to the lack of an endogenous ethanol production pathway and wax ester synthase (AftA) activity in the wild-type cyanobacterium, we metabolically engineered *S. elongatus* PCC 7942 by expressing heterologous AftA and introducing the ethanol pathway, resulting in detectable peaks of FAEEs. To enhance FAEE production, a heterologous phosphoketolase pathway was introduced in the FAEE-producing strain to supply acetyl-CoA. Subsequent optimization of the cyanobacterial

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culture with a hexadecane overlay resulted in engineered *S. elongatus* PCC 7942 that produced photosynthetic FAEEs (10.0 ± 0.7 mg/L/OD_{730}) from CO_2. This paper is the first report of photosynthetic production of FAEEs from CO_2 in cyanobacteria.

Industrial Applications

Enhancing the productivity of batch deodorizers for edible oils

Laoretani, D.S. and O.A. Iribarren, *J. Food Eng. 192*: 72–78, 2017, http://dx.doi.org/10.1016/j.jfoodeng.2016.08.004.

This paper addresses the potential of a process alternative aimed at improving the efficiency of batch deodorizers, coupling them to a small continuous desorption packed column: The steam exiting the batch deodorizer is fed to the bottom of the column, while the oil contained in the batch vessel is recycled through the top of the column and then returned to the vessel. This strongly increases the efficiency of separation, reducing stripping steam consumption by 16.5% and processing time by almost a half, from 3.0 h to 1.8 h. The required additional equipment consists of a small column section and a pump, that increase the cost of investment by 22% compared to conventional batch. Thus, the alternative here proposed achieved a pretty good preliminary economic assessment with a positive profit of $61,970/year) above the conventional batch process. Overall, the semi-batch design proved to have a better performance than the batch mode in both economic and flexibility terms, while continuous is far better in economics but far worse in flexibility than both batch designs.

A review on latest developments and future prospects of heterogeneous catalyst in biodiesel production from non-edible oils

Mardhiah, H.H., *et al.*, *Renew. Sust. Energ. Rev. 67*: 1225–1236, 2017, http://dx.doi.org/10.1016/j.rser.2016.09.036.

Research on biodiesel production via a heterogeneous catalyzed approach is focused on sustainability and quality. The sources of the feedstock and the catalyst are major factors that influence the yield and sustainability of the process. The easy separation and high reusability of heterogeneous catalysts offer a simple and low cost manufacturing process. However, the most distinguishing characteristic of heterogeneous catalysts is that their properties can be tuned to generate acidic-basicity, surface area, and porosity. Such tuning makes it possible to use a wider variety of feedstocks for esterification/transesterification processes. The traits of various heterogeneous catalysts (solid base, solid acid, acid-base and biocatalyst) are studied in this review, as each offers specialty features. The use of non-edible feedstocks is discussed as it relates to the food vs. fuel debate and low-cost production.

Intensification of biodiesel production using hydrodynamic cavitation based on high speed homogenizer

Mohod, A.V., *et al.*, *Chem. Eng. J.* online February 2017, http://dx.doi.org/10.1016/j.cej.2017.02.011.

Biodiesel offers as an excellent alternative to petro-based diesel fuel, and can be derived from the reaction of vegetable/non-edible oils and/or animal fats with alcohols using the transesterification reaction. In this study, a High Speed Homogenizer was used as a cavitation device for the intensified production of biodiesel for the first time. The efficacy of biodiesel production was observed to be dependent on the operational parameters viz. molar ratio, catalyst loading and operating temperature. The maximum yield of biodiesel obtained in the present work was 97% for waste cooking oil as starting material and 92.3% for fresh cooking oil under optimized conditions of reaction time of 120 min, molar ratio of methanol to oil as 12:1, 3% wt loading of KOH, and temperature of 50°C. The study demonstrated that the application of cavitation can enhance the progress and speed of reaction, and improve separation. Overall, high speed homogenizer was demonstrated to be a viable approach for intensified biodiesel production with possibly favorable economics.

Biodiesel production using mobile processing units: a case in Indonesia

ten Kate, J., *et al.*, *Agr. Syst. 152*: 121–130, 2017, http://dx.doi.org/10.1016/j.agsy.2016.12.015.

Biodiesel is a sustainable alternative to fossil fuel, particularly if the input materials are not edible. This paper considers the production of biodiesel from rubber seeds, which are currently viewed as a waste product and discarded by farmers. We investigate whether technological innovations in local pre-processing at individual farms, and oil production via mobile processing units (MPUs) visiting villages makes bio-diesel production profitable for groups of farmers. A mixed integer programming model optimizing supply chain decisions is developed and applied to a specific case in Indonesia. We find that operating the relatively expensive MPUs at maximum capacity is essential for economic sustainability. Furthermore, variations in the price of biodiesel affect the profitability and governments may consider offering minimum price guarantees.

Supercritical CO_2 oilseed extraction in multi-vessel plants. 3. Effect of extraction pressure and plant size on production cost

Núñez, G.A., *et al.*, *J. Supercrit. Fluids 122*: 109–118, 2017, http://dx.doi.org/10.1016/j.supflu.2016.11.002.

This work completes a series of three studies that estimated the cost of extracting vegetable oil from seeds using supercritical CO_2. The focus of this work was to determine the effect of

extraction pressure (30, 50, and 70 MPa) on the production cost of a supercritical CO_2 extraction of a packed bed of 2-mm oilseeds in plants with two-extraction vessels with aspect ratio of 4.5, using a superficial velocity of 5 mm/s. Increasing extraction pressure while keeping other variables constant decreased the production cost. For 2 × 1 m^3 extraction plants, increasing extraction pressure from 30 to 50 MPa decreased the production cost by 30.9%, but increasing extraction pressure from 50 to 70 MPa only further decreased the production cost by 9.9%. Results suggest that the recommended extraction pressure for oilseed extraction is 50 MPa. This work also compared plants operating at the three extraction pressures when annual productivity was kept constant (231, 456, or 596 tons of oil per year). The total extraction volume of the plant diminished as the extraction pressure increased. The optimum production cost in these cases was quite similar for plants operating at 30, 50, and 70 MPa, but the total volume of the plant decreased 63.6% when extraction pressure was increased from 30 to 50 MPa for the plant producing 456 ton of oil per year, and an additional 31% when it was increased from 50 to 70 MPa. The lowest production cost estimated was 5.52 USD per kg of oil (optimum extraction time of 2.75 h) in a 4 × 1 m^3 plant processing 2-mm prepressed oilseeds, and operating at 40 °C and 50 MPa. The manuscript includes a sensitivity analysis on production cost as a function of a variation of ±50% of uncertain variables cost (respect to nominal values), which were considered as constant in our previous contributions. Given that cost reflects unitary costs of annual labor, CO_2, and substrate, it was determined that varying the substrate had a more significant effect on the production cost.

Simulation and process design of continuous countercurrent ethanolic extraction of rice bran oil

Bessa, L.C.B.A., *et al.*, *J. Food Eng. 202*: 99–113, 2017, http://dx.doi.org/10.1016/j.jfoodeng.2017.01.019

The overwhelming majority of vegetable oils are extracted using solvent extraction, and the most widely used solvent is hexane. However, the use of ethanol has attractive advantages, including low toxicity, good operational security, and bio-sourcing. In this study, a multiple-batch, solid-liquid extraction system was successfully employed to simulate continuous countercurrent ethanolic extraction of rice bran oil. Results showed that the extraction process using ethanol as solvent is feasible and facilitated by increases in temperature. The number of equilibrium stages required for rice bran oil extraction was theoretically determined, and it was shown that in the extraction process using hexane the number of ideal stages is lower than the number required when ethanol is used as solvent. Furthermore, a mathematical expression for determining the minimum flow of solvent depending on the equilibrium relationship between the extract and raffinate phases has been developed and implemented for both solvents. Given the potential fire hazard and harmful emissions risks of using hexane as solvent, extraction with ethanol showed to be a promising alternative to conventional extraction, completely exhausting the solid matrix with only five stages.

Synthetic Biology

Engineering *Yarrowia lipolytica* for arachidonic acid production through rapid assembly of metabolic pathway

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Yarrowia lipolytica, a non-conventional oleaginous yeast with special traits, has attracted increasing interest for producing value-added products. Generally, the DNA fragments of these heterologous metabolic pathways are constructed *via* the classic restriction digestion and ligation method. In contrast, the one-step *in vivo* pathway assembly method has been only rarely applied to *Y. lipolytica*. Here, with arachidonic acid biosynthesis as a case study, a one-step *in vivo* pathway assembly and integration method was used for engineering *Y. lipolytica*. Using rDNA as integrative locus, this study showed that there was a relation between the assembly efficiency and the length of overlapping region. Especially, with an overlap up to 1 kb, the method was able to rapidly assemble the arachidonic acid biosynthesis pathway (nearly 10 kb) into the chromosome with high efficiency (nearly 23%). Meanwhile, the pathway assembled in *Y. lipolytica* demonstrated long-term genetic stability and the engineered strain exhibited robust growth. Furthermore, this study demonstrated that the codon-optimized genes from *Mortierella alpina* can function efficiently in *Y. lipolytica*: a high level arachidonic acid production (0.4% of total fatty acids) was produced in the engineered strain. To our knowledge, this is the first time that this method is applied to *Y. lipolytica* for functional polyunsaturated fatty acids production. This method represents a powerful tool with potential for facilitating engineering applications in non-conventional yeasts.