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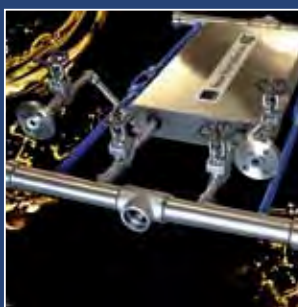
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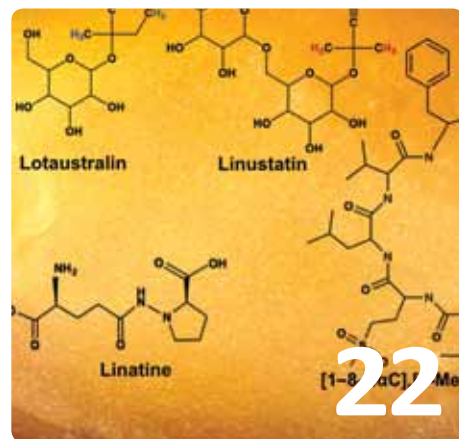
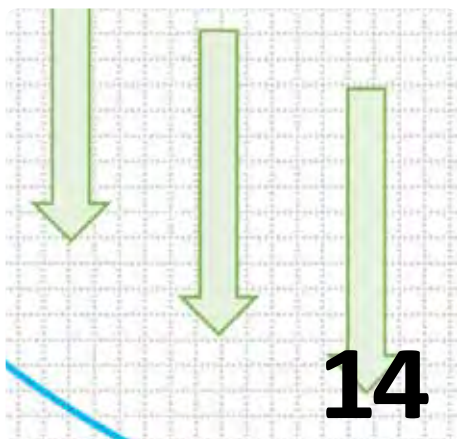
April 2016

INFORM

CONTENTS

6 Sink or swim: fish oil supplements and human health

Mixed clinical trial results leave researchers divided on the health benefits of omega-3 fatty acid supplements.



14

TD-NMR and chemometrics for fat analysis in food through packaging

Time-domain nuclear magnetic resonance (TD-NMR) is a promising technique for measuring fat content through packaging.

18

Critical design phases for upgrading oilseeds facilities

Considering a facility upgrade? Use the step-by-step process outlined in this article to guide you through the five critical design phases.

22

The flaxseed portfolio of natural health ingredients

Can flaxseed production and consumption be expanded as a source of ω -3 oils? This article looks at the nutraceutical and functional aspects of flaxseed and highlights several commercial products.

26

Multi-spectral hand-held devices for edible oil and general food inspection

Autonomous, multi-sensory, wireless measurement systems incorporated into kitchen utensils make it possible for ordinary consumers to distinguish a range of ingredients from one another and from adulterated samples.

30

Top grade oil

Learn how seed quality affects the 11 steps used to process canola as well as the final quality of the refined oil and meal pellets.

52

Pennycress: What's the catch?

Field pennycress has not lived up to its potential, but scientists are using advanced breeding technology to improve the plant's viability as a biofuels feedstock.

61

Method of producing DHA-rich oil using lipase

Researchers describe how they used lipase produced by *Candida rugosa* to produce a DHA-rich oil that is lower in saturated fats and cholesterol.

DEPARTMENTS

5 Index to Advertisers
40 Classified Advertising
58 AOCS Meeting Watch

Analysis/commentary
34 Olio
54 Latin America Update
57 Regulatory Review

Publications and more
37 AOCS Journal Titles
39 Patents
41 Tips from
inform|connect
42 Extracts & Distillates



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INDEX TO ADVERTISERS

*Avanti Polar Lipids, Inc.	41
*Bruker Optics	33
Buhler, Inc.	51
*Corbion Caravan	4
*Crown Iron Works Company.....	C3
*Desmet Ballestra Engineering NA.....	C2
DVC Process Technologists	49
*French Oil Mill Machinery Co.	31
Kumar Metal Industries Pvt. Ltd.	48
Myers Vacuum, Inc.	21
*Oil-Dri Corporation of America.....	C4
Pope Scientific, Inc.	9
Sharplex Filters (India) Pvt. Ltd.	13
Solex Thermal Science, Inc.	17
Veendeep Oilteck Experts Pvt. Ltd.	46

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Sink or swim: fish oil supplements and human health

Laura Cassiday

In 1970, two Danish researchers heard what sounded like a fish tale: Despite consuming a high-fat diet consisting mainly of seal and whale meat and blubber, the Inuit people of northern Greenland had a remarkably low rate of coronary artery disease (CAD) and almost nonexistent diabetes mellitus. Highly carnivorous, the traditional Inuit diet supplies about 280 grams of animal protein and 135 grams of fat per day, with few or no vegetables (Bang, H. O., *et al.*, *Lancet*, 1971)—in other words, exactly the opposite of what most nutritionists recommend.

- The omega-3 fatty acids DHA and EPA have physiological effects that may improve cardiovascular and neurocognitive health.
- Clinical trials of fish oil supplements containing DHA and EPA have produced mixed results.
- Researchers are still divided on whether omega-3 fatty acid supplements are beneficial, neutral, or even harmful to human health.

Intrigued by these reports, Hans Olaf Bang and Jørn Dyerberg mounted an expedition to the northwest coast of Greenland, traveling across an ice sheet by dog sled to reach a remote village of approximately 1,350 Inuit. The researchers collected and analyzed blood samples from 61 male and 69 female Inuit and compared their plasma lipid profiles to those of healthy Danes. Their finding: The Inuit had lower levels of several types of lipids, including total cholesterol and plasma triglycerides, than Danish controls (Bang, H. O., *et al.*, *Lancet*, 1971). Bang and Dyerberg later discovered that the Inuit had higher-than-normal amounts of two omega-3 fatty acids—docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3)—in their plasma and platelet lipids that increased blood clotting time, leading the researchers to hypothesize that omega-3s could protect the Inuit from the cardiovascular consequences of their high-fat diet (Dyerberg, J., and Bang, H. O., *Lancet*, 1979).

Bang and Dyerberg's hypothesis spawned an entire industry that sought to encapsulate the protective components of the Inuit diet in a convenient pill that would obviate the need to consume seal or whale blubber or even fish, which many people find unpalatable. Omega-3s were hailed as the panacea for all of Western society's ills, from cardiovascular disease to cancer to cognitive decline. Although early clinical trials seemed to reinforce the cardioprotective effects of omega-3s, more recent trials have produced mixed results, at best. Some researchers have even suggested that a high intake of omega-3s could be harmful to certain populations.

A 2014 report questioned the entire premise behind Bang and Dyerberg's work, claiming that the Danish researchers vastly underestimated the prevalence of CAD in the Inuit (Fodor, J. G., *et al.*, <http://dx.doi.org/10.1016/j.cjca.2014.04.007>). According to the study, not only did Inuit in the 1970s have rates of CAD similar to or greater than non-Inuit populations, they also had excessive mortality from stroke and an overall mortality rate twice as high as that of non-Inuit. "Considering the dismal health status of Eskimos [Inuit], it is remarkable that instead of labeling their diet as dangerous to health, a hypothesis has been construed that dietary intake of marine fats prevents CAD and reduces atherosclerotic burden," the researchers write.



Nevertheless, Bang and Dyerberg's studies set a ball rolling that culminated in a large and lucrative omega-3 supplement industry. According to a recent market research report (Packaged Facts; Rockville, Maryland, USA), the market value of EPA + DHA packaged products is projected to reach \$34.7 billion in 2016, with a compound annual growth rate of 6.4% from 2011 (<http://tinyurl.com/omega3market>). Fish oil, which contains a mixture of DHA, EPA, and other fatty acids extracted from fish, is now the most popular nonvitamin, nonmineral supplement in the United States, taken by approximately 7.8% of adults in 2012 (up from 4.8% in 2007; <http://tinyurl.com/cdc-omega-report>). Between 2005 and 2012, fish oils sales more than doubled worldwide (O'Connor, A., *The New York Times*, <http://tinyurl.com/fishoilclaims>, 2015).

ESSENTIAL OILS

Enzymes in the human body can synthesize low levels of EPA and DHA from α -linoleic acid (ALA, C18:3n-3) consumed in the diet from plant-derived foods, such as canola and soybean oils and walnuts. However, the process is very inefficient because ALA competes with linoleic acid (an omega-6 fatty acid) for the same enzymes. Because the human body cannot make ALA or linoleic acid, they are both essential fatty acids that must be obtained through the diet or supplements.

Other enzymes convert EPA and DHA into eicosanoids such as prostaglandins, thromboxanes, and leukotrienes—signaling molecules that have potent anti-inflammatory, anti-thrombotic, antiarrhythmic, and vasodilatory effects (Jain, A. P., *et al.*, *Eur. Rev. Med. Pharmacol. Sci.*, 2015). In contrast, omega-6 fatty acids such as linoleic acid are converted into arachidonic acid (C20:4n-6), a precursor to different eicosanoids that are pro-inflammatory and pro-thrombotic. Vegetable oils such as corn and sunflower are high in omega-6 fatty acids. Increasing consumption of vegetable oils in the past century has shifted omega-6:omega-3 ratios from 1:1 in 1900 to 15:1 in Europe and 25:1 in the United States, according to Paul Clayton, fellow at the Institute of Food, Brain & Behav-

ior in Oxford, UK. "If you have a diet that is excessively loaded with omega-6s, creating an excessive omega-6 to omega-3 ratio, then you start producing a much more toxic mix of pro-inflammatory lipid mediators," Clayton says. Many researchers believe that this drastic dietary change underlies the surge in diseases characterized by chronic inflammation, such as atherosclerosis, type 2 diabetes, and cancer.

DHA and EPA consumed in the diet or through supplements are key components of cell membranes. Neuronal membranes are particularly enriched in DHA, leading researchers to propose that the omega-3 fatty acid is important for brain health. Although DHA is typically present at much higher levels in cell membranes than EPA, both omega-3s can bind to membrane-bound proteins and block ion channels. Omega-3s can also enter cells and interact with enzymes and transcription factors to alter metabolism.

SUCCESS STORIES

Three large randomized, controlled clinical trials of fish or fish oil supplements for the treatment of cardiovascular disease bolstered the enthusiasm for omega-3s as cardioprotective agents. The Diet and Reinfarction Trial (DART) study followed for two years 2,033 men who had experienced myocardial infarction (Burr, M. L., *et al.*, *Lancet*, 1989). Men who were instructed to consume at least two meals of coldwater fatty fish (for example, mackerel, sardine, or salmon; corresponding to 500–800 mg DHA/EPA per day) per week had a 32% reduced incidence of reinfarction and a 29% reduction in all-cause mortality, compared with a control group that received no instructions to eat fish.

Another major study, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione, randomized 11,323 survivors of myocardial infarction to two groups: one that received a combined EPA/DHA dose of 1 gram from fish oil supplements, and a control group that received regular care (Marchioli, R., *et al.*, *Circulation*, 2002). After only four months of treatment, the fish oil group had

a 28% reduced risk of death from any cause, driven mainly by a 45% reduced risk of sudden cardiac death. The differences between the groups remained significant throughout the 3.5 years of the study.

The Japan EPA Lipid Intervention Study (JELIS) demonstrated that EPA supplementation alone could have beneficial effects, even in a population that has a high background consumption of fish (Yokoyama, M., *et al.*, [http://dx.doi.org/10.1016/S0140-6736\(07\)60527-3](http://dx.doi.org/10.1016/S0140-6736(07)60527-3), 2007). JELIS followed 18,645 patients with hypercholesterolemia, who were assigned to groups receiving statin treatment alone, or a combination of statin and 1.8g EPA. After five years, the combination treatment was associated with a 19% reduced risk of major coronary events in patients with a history of CAD compared with the statin-only group. In contrast, EPA treatment was not associated with a reduced risk of sudden cardiac death.

Another study has shown that the risk of sudden cardiac death increases with decreasing blood levels of omega-3s (Albert, C. M., *et al.*, *N. Engl. J. Med.*, 2002). Moreover, the amount of DHA in plasma and cellular phospholipids, which closely correlates with the amount of DHA in heart muscle, is inversely related to the risk of CAD events (Harris, W. S., *Pharmacol. Res.*, 2007). As a result of these findings, Harris has proposed a new marker for cardiovascular risk—the omega-3 index—that reflects the proportion of omega-3 fatty acids in the membranes of red blood cells. An omega-3 index greater than 8% is associated with the lowest risk of cardiovascular events, whereas an index less than 4% is typically found in CAD patients.

DOSE MATTERS

Because most clinical trials have focused on patients who already have a history of cardiovascular disease or are at high risk, evidence for benefits of omega-3 fatty acids is stronger for secondary prevention than primary prevention. The American Heart Association (AHA) recommends two servings of fatty coldwater fish per week for people with no history of CAD, or one serving per day for people with CAD. In 2002, the AHA endorsed omega-3 supplements for the secondary prevention of heart disease (HD), while stating that a food-based approach (i.e., eating fatty fish) is preferable (Kris-Etherton, P. M., *et al.*, *Circulation*, 2002). In terms of omega-3 amounts, 300–600 mg/day combined DHA/EPA are recommended for people without a history of CAD, 900–1,200 mg/day for people with a CAD history, and 3,000–4,000 mg/day for triglyceride lowering. Different dosages of DHA/EPA may confer different cardiovascular benefits (Lee, J. H., *et al.*, <http://dx.doi.org/10.4065/83.3.324>, 2008) (Table 1). For example, low doses (0.5–1.0 g/day DHA/EPA) can lower the risk of sudden cardiac death in people with CAD, while very high doses (8.0 g/day) can reduce inflammation and decrease body fat in patients with heart failure. In addition, the time course of different benefits may vary. With the appropriate dose of DHA + EPA, patients can achieve strong antiarrhythmic

benefits in a matter of weeks, compared with months to years for triglyceride or blood pressure lowering.

Relatively high doses (3–4 g/day) of omega-3s can lower triglyceride levels by 30–50% (Lee, J. H., *et al.*, <http://dx.doi.org/10.4065/83.3.324>, 2008). When added to statin therapy, DHA + EPA reduces triglycerides by an additional 23–29% compared with statins alone. The US Food and Drug Administration (FDA) has approved three prescription drugs that deliver concentrated fish oil for the treatment of very high triglycerides.

SOMETHING FISHY

Beginning in the mid-2000s, various clinical trials and meta-analyses failed to confirm the earlier successes of DART, GISSI-Prevenzione, and JELIS. Between 2005 and 2012, more than 24 studies of fish oil supplements were published in respected medical journals, most examining whether fish oil could prevent cardiovascular disease in people at high risk (O'Connor, A., *The New York Times*, 2015). All but two of the studies found no benefit of fish oil compared with placebo.

In 2012, a meta-analysis of 20 fish oil trials including a total of 68,680 patients found no association between omega-3 supplementation and lowered risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke (Rizos, E. C., *et al.*, <http://dx.doi.org/10.1001/2012.jama.11374>). Similarly, a 2013 randomized controlled trial of 12,513 people found that 1.0 g/day of fish oil did not reduce the rate of death from cardiovascular disease or the risk of hospitalization from

TABLE 1. Doses of omega-3 fatty acids shown to have cardioprotective effects

Effect	Dose of omega-3
Reduced risk of sudden cardiac death in people with CAD	0.5–1.0 g/day of DHA + EPA*
Decreased resting heart rate, increased post-exercise heart rate recovery in people with CAD	0.81 g/day of DHA + EPA
Increased level of adiponectin in obese individuals	1.8 g/day EPA
Antiplatelet, anti-inflammatory, triglyceride-lowering effects	3.0–4.0 g/day of DHA + EPA
Reduced systolic and diastolic blood pressure	4.0 g/day DHA + EPA
Anti-inflammatory effects, improved body composition in people with heart failure	8.0 g/day DHA + EPA

*Refers to combined dose of DHA and EPA.

Credit: Adapted from information in Lee, J. H., <http://dx.doi.org/10.4065/83.3.324>, 2008.

cardiovascular causes in people who had a history of CAD (Roncaglioni, *et al.*, <http://dx.doi.org/10.1056/NEJMoa1205409>). “Our findings provide no evidence of the usefulness of n-3 fatty acids for preventing cardiovascular death or disease in this population,” the researchers concluded.

Scientists have proposed several reasons for the discrepancies among fish oil trials. One possible explanation is that the standard of care for CAD has increased over the years, so that in the earlier studies, even a minor effect of fish oil would have been more noticeable. For example, in DART no patients received statin therapy, and in GISSI-Prevenzione, only 5% did. “Back then they didn’t use the cocktail of drugs they use today,” says Adam Ismail, executive director of the Global Organization for EPA and DHA Omega-3s (GOED), a not-for-profit trade association based in Salt Lake City, Utah, USA. “But today, 100% of the patients in secondary prevention studies are on at least one medicine, and more than 75% are on three to five medicines such as ACE inhibitors, beta blockers, aspirin, and statin drugs. As the standard of care increased, that’s really when you saw the change in success rates of these trials.”

“I don’t think our treatments have become substantially better,” disagrees Paul Clayton. “I think it is far more likely that the reason why current studies aren’t as successful is because the effect of omega-3 supplements depends on the extent to which you can reduce the omega-6 to omega-3 ratio. We know from studies that have been published in different ages that the 6-to-3 ratio has worsened considerably over the last few decades.”

According to Clayton, nutrition researchers need to stop looking for a magic bullet to treat disease, but instead target the real culprit in many ailments: chronic inflammation. “The idea that you can interfere with neurological disease, cancer, or diabetes with a couple of fish oil capsules is frankly laughable,” remarks Clayton. “It says more about the tunnel vision of the pharmaceutically trained doctors who are doing these studies than it has anything to do with nutritional and clinical reality.” Another possible explanation for unsuccessful omega-3 trials is suboptimal dosage. “I’m kind of baffled by the approach a lot researchers are taking,” says Ismail. “You’ve got populations that are suffering from coronary heart disease, and yet in many of these studies you’re seeing dosages that are less than the American Heart Association recommendation of 1.0 gram per day of omega-3s for secondary prevention.” A recent meta-analysis examined 11 randomized controlled trials from 1995 to 2013, each of which treated patients with existing cardiovascular disease with at least 1 g/day of omega-3 supplements for at least one year (Casula, M., *et al.*, [http://dx.doi.org/10.1016/S1567-5688\(13\)70005-9](http://dx.doi.org/10.1016/S1567-5688(13)70005-9), 2013). In contrast to meta-analyses that included studies with lower doses or shorter durations, this analysis showed a 32% reduced risk of cardiac death, a 33% reduced risk of sudden death, and a 25% reduced risk of myocardial infarction for people taking omega-3 supplements compared with placebo.

BRAIN FOOD

In addition to conferring possible cardiovascular benefits, omega-3 fatty acids have been proposed to help sharpen memory and to prevent cognitive decline, dementia, and neurodegenerative disease. Indeed, DHA is an essential structural component of neurons, comprising more than 40% of polyunsaturated fatty acids in the brain. Epidemiological studies have indicated that diets high in omega-3 fatty acids protect cognitive function. Also, people with Alzheimer’s disease have reduced serum levels of DHA. However, several randomized controlled trials have failed to demonstrate the effectiveness of omega-3 supplements for treating dementia.

The Age-Related Eye Disease Study 2 (AREDS2) examined the effects of omega-3 and other supplements on age-related macular degeneration (AMD) and cognitive decline (Chew, E. Y., *et al.*, <http://dx.doi.org/10.1001/jama.2015.9677>, 2015). Like brain cells, retinal cells are highly enriched in DHA. The researchers treated 4,203 elderly patients (mean age at baseline, 72.7 years) at high risk of developing late AMD with 350 mg DHA and 650 mg EPA. Over the course of the five-year study, the participants were given cognitive function tests by telephone, which included such tests as immediate and delayed recall of 10 words and counting backwards from 100 as quickly as possible. The researchers found that test scores for both the omega-3 and placebo groups declined to the same extent with time. “Contrary to popular

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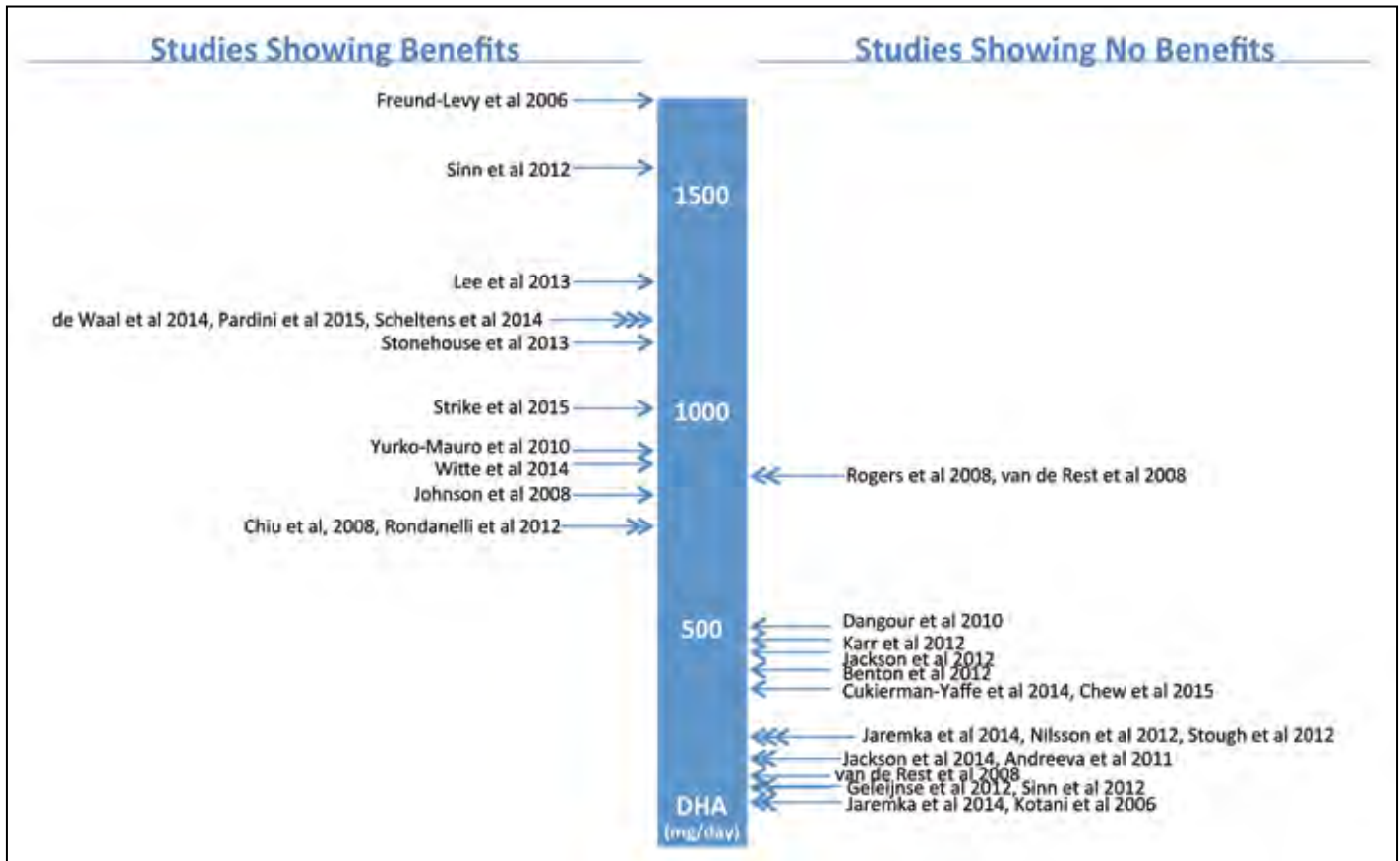


FIG. 1. Omega-3 cognitive function studies and their outcomes sorted by DHA dosage.

Credit: Adam Ismail, GOED

belief, we didn't see any benefit of omega-3 supplements for stopping cognitive decline," says lead author Emily Chew of the National Eye Institute, part of the National Institutes of Health (Bethesda, Maryland, USA). Nor did the researchers see benefits of any supplement that they tested for either cognitive decline or AMD.

A recent meta-analysis of six cohort studies with a total of 22,402 participants likewise failed to detect an association between fish oil intake and the risk of dementia or Alzheimer's disease (Wu, S., *et al.*, <http://dx.doi.org/10.1016/j.neubio-rev.2014.11.008>, 2015). However, a higher intake of fish was associated with a 36% lower risk of Alzheimer's disease. For each 100 g of fish eaten per week, there was an 11% reduced risk of Alzheimer's.

Ismail suspects that negative results for neurocognitive studies may be explained by insufficient dosages of DHA. A 2015 meta-analysis indicated that greater than 1.0 g of DHA per day was required to improve memory in adults (Yurko-Mauro, K., *et al.*, <http://dx.doi.org/10.1371/journal.pone.0120391>). "There is a cutoff where every single study conducted with over one gram of DHA has found a benefit on cognitive function," says Ismail. "And almost every single study that had less than one gram found no benefit." (Fig. 1)

"Obviously, when there's not an effect, you are always concerned that you didn't have the right dose of EPA or DHA, or it was in the wrong ratio, but we point out these limitations in our

paper," says Chew. "Also, our patients are much older, with an average age of 73, so it may be that we are getting in the game too late." Perhaps starting omega-3 supplementation at an earlier age could have an effect on cognitive decline, she says.

MORE HARM THAN GOOD?

Some researchers have hypothesized that instead of being beneficial, omega-3 supplements may actually be harmful to human health because the polyunsaturated fatty acids are highly susceptible to oxidation (Albert, B. B., *et al.*, <http://dx.doi.org/10.1155/2013/464921>, 2013). Omega-3s contain multiple double bonds and bisallylic carbons (carbon atoms between two double-bonded carbon atoms), which make them prone to hydrogen loss and free radical formation. In a chain reaction, the lipid radical can generate lipid peroxides and more radicals from unoxidized polyunsaturated fatty acids. Lipid peroxides then degrade into secondary oxidation products—aldehydes such as 4-hydroxyhexanol and malondialdehyde. These primary and secondary oxidation products can damage cellular membranes, proteins, and DNA.

During the manufacture of fish oil, the deodorization process that removes the fishy odor often involves high temperatures, which may accelerate secondary oxidation. Antioxidants, most commonly vitamin E, added to fish oil can reduce but not prevent oxidation. As a result, fish oil supple-

ments are a complex mix of EPA, DHA, other fatty acids, additives, and potentially toxic lipid peroxides and secondary oxidation products. Studies have shown that the frequency of excess oxidation in over-the-counter fish oil supplements is variable, affecting 11–62% of products (Albert, B. B., *et al.*, <http://dx.doi.org/10.1155/2013/464921>, 2013).

Animal studies indicate that the consumption of oxidized lipids is harmful, but usually at higher doses of oil than humans consume. One human randomized controlled trial examined the effects of oxidized versus nonoxidized oil over seven weeks (Ottestad, I., *et al.*, <http://dx.doi.org/10.1017/S0007114511005484>, 2012). Reassuringly, perhaps, the researchers found no difference in markers of *in vivo* lipid peroxidation, such as antioxidant activity, C-reactive protein, or liver function. However, the study was of short duration and did not assess other markers of atherosclerosis, inflammation, or DNA damage. “In summary, given the paucity of specific evidence, it is currently impossible to know whether marine oils, some of the world’s most popular supplements, are safe after oxidation,” Albert *et al.* write (<http://dx.doi.org/10.1155/2013/464921>, 2013).

There is increasing evidence that *in vivo* oxidation of LDL plays a role in atherogenesis. If this is correct, oxidized supplements could help explain the disappointing results of some primary and secondary cardiovascular prevention trials. According to Albert *et al.* (<http://dx.doi.org/10.1155/2013/464921>, 2013), clinical trials should analyze and report the oxidative status of trial oils, so that the benefits and harms could be associated with the oil’s oxidative state. The researchers say that the oxidation status can be easily estimated using two assays: the peroxide value (PV; a titration that enables quantification of peroxide groups) and the anisidine value (AV; a colorimetric test that allows estimation of secondary oxidation products). Total oxidation of the oil can be estimated by the formula: TOTOX = 2PV + AV.

“We do think oxidation is an issue, but it’s really a sensory issue more than anything because if a product is oxidized, it’s going to taste fishy, and consumers won’t want to take the product,” says Ismail. He notes that the fish oil industry has set voluntary limits on oxidation that are much lower than those for vegetable oils. “Our limit for peroxide value is 5, for refined vegetable oils it’s 10, and for extra virgin olive oil it’s 20,” he says. In collaboration with the Council for Responsible Nutrition (Washington, D.C., USA), GOED recently published a white paper on oxidation in omega-3 oils (<http://tinyurl.com/GOEDwhite>, 2015). The paper points out that the anisidine value is not applicable to omega-3 products that contain added flavorings or those with natural pigments, such as krill oil. In an analysis of more than 2,000 test results from the scientific literature, third-party testing labs, and GOED’s industry testing program, the authors found that greater than 94% of omega-3 products met the voluntary GOED limit for peroxide value, and almost 98% met the limit for anisidine value.

OPTIMIZING OMEGA-3s

Because of conflicting clinical trials, researchers and health professionals are divided on whether fish oil is truly beneficial to health. However, eating whole fish has consistently been shown to benefit cardiovascular health in epidemiological and observational studies (long-term randomized, controlled dietary trials are logistically difficult). It is possible that factors in fish other than omega-3s, such as other nutrients or antioxidants, may contribute to this cardioprotective effect. Alternatively, people who consistently eat fish may tend to have a higher socioeconomic status or a more healthful diet in general.

“I think that the current fish oil industry is selling nothing but hope,” says Clayton. “I think their fundamental premise is faulty. It was never just about omega-3s.” Instead, Clayton believes that antioxidants known as lipophilic polyphenols, which are removed or destroyed during fish oil processing, contribute to fish’s health benefits (Clayton, P. R., and Ladi, S., <http://dx.doi.org/10.1177/0141076815599673>, 2015). Lipophilic polyphenols such as phlorotannins from oily fish and secoiridoids from olives can protect omega-3s and other lipids from oxidation within the body. The antioxidants also exert their own anti-inflammatory effects, such as inhibiting matrix metalloproteinases in the arterial wall. Their lipophilic nature favors partitioning into adipose tissue, where they may inhibit the formation of pro-inflammatory cytokines.

Clayton and his colleagues have treated thousands of patients with combinations of omega-3s and secoroidoids. “Whereas the picture with the omega-3s is, to put it mildly, diverse, when we give patients combinations of omega-3s and these lipophilic polyphenols, chronic inflammation in the body, wherever it is, just seems to stop,” says Clayton. He emphasizes that these studies are for now only case histories, and thus “scientifically worthless,” but the researchers plan to conduct a randomized controlled trial of omega-3s and lipophilic polyphenols in 2016.

Another unresolved question is whether oil extracted from fish is the best source of omega-3s. DHA and EPA in fish oil exist mainly as triglycerides, whereas in krill oil they are incorporated into phospholipids. Krill are shrimp-like crustaceans that feed on the algae that produce omega-3s. In turn, fish eat krill, and omega-3s accumulate up the trophic chain. Limited studies conducted so far suggest that DHA and EPA in phospholipid form may be more bioavailable than the omega-3s in triglyceride form (Ulven, S. M., and Holven, K. B., <http://dx.doi.org/10.2147/VHRM.S85165>, 2015). Phospholipids may help omega-3s enter the lipid bilayer of cells and pass through the intestinal wall. Krill oil also contains the antioxidant astaxanthin, which may protect fatty acids from oxidative damage.

However, krill oil has an EPA:DHA ratio of 2:1, whereas fish oil has a ratio of 1:1. Because EPA and DHA give rise to different metabolites, the two omega-3 sources may have different physiological effects. Indeed, gene expression data from animal studies has shown that fish oil and krill oil regulate different metabolic pathways (Ulven, S. M., and Holven, K. B., <http://dx.doi.org/10.2147/VHRM.S85165>, 2015). Researchers have also explored the use of algae as a source for omega-3s.

CONTINUED FROM PAGE 11

Algae represent a more environmentally sustainable source of omega-3s than either fish or krill, and different algal strains can produce oils with different EPA:DHA ratios.

While consuming fish may be best, “the problem is that there are not enough oily fish to feed everyone, and many people don’t like them,” says Clayton. In addition, some health experts are concerned over levels of methyl mercury and other toxic contaminants in fish. Because mercury is bound to protein and is water soluble, fish oil supplements contain negligible amounts of the toxin.

THE INUIT ENIGMA

Since Dyerberg and Bang’s studies of the Inuit in the 1970s, thousands of papers have been published on the effects of omega-3 fatty acids on human health. Despite mixed results in clinical trials, an increasing number of people worldwide consume fish oil supplements. Whether improved formulations or optimized doses of fish oil or other marine oils will ultimately prove beneficial for human health remains to be seen.

In an interesting twist to the Inuit story, researchers led by Rasmus Nielsen at the University of California–Berkeley (USA) recently reported in *Science* that the Inuit have accumulated genetic mutations that may have helped them adapt to a diet high in omega-3s (Fumagalli, M., *et al.*, <http://dx.doi.org/10.1126/science.aab2319>, 2015). “We were interested in finding genes in the genome of the Inuit that have evolved very fast and ultimately become different from other populations,” says Nielsen. “We were searching for the traces of natural selection.”

After sequencing the DNA of several thousand Inuit and comparing to control populations, Nielsen and his colleagues identified several genome regions that differ in Inuit compared to Europeans or Chinese. The most tantalizing of these regions corresponded to genes that encode fatty acid desaturases (FADS). FADS2 is an enzyme that converts ALA from the diet into EPA and DHA. “These genes have been down-regulated in the Inuit so that they now naturally produce less omega-3 fatty acids,” says Nielsen. “Our hypothesis is that this is an adaptation to a diet rich in omega-3 fatty acids. The Inuit get so many in their diet that they produce fewer themselves.”

These findings may have important implications if, as some researchers suspect, high levels of omega-3 fatty acids can be harmful due to lipid oxidation. However, Nielsen stresses that his study cannot provide any specific guidelines regarding nutrition. “I think our study may provide a little piece of the puzzle in that it appears that you cannot extrapolate from the Inuit to other populations,” he says. “It could be very good for the Inuit to eat all these omega-3 fatty acids, but not for the rest of us.”

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TD-NMR and chemometrics for fat analysis in food through packaging

In food science, fat content determination is usually performed with standard methods of wet chemical analysis. Such analyses sometimes use toxic organic solvents and consequently generate toxic waste. They are also slow and destructive, and cannot be applied to every product.

Fabíola Manhas Verbi Pereira

- The standard methods of wet chemical analysis that are used to determine fat content in foods generate toxic waste, are slow and destructive, and cannot be applied to every product.
- Time-domain nuclear magnetic resonance (TD-NMR) was recently demonstrated to be a promising non-invasive technique for measuring fat content through packaging—with almost no limitations on sample size, and without weighing the samples.
- This article describes the use of the TD-NMR signals profile and the chemometrics approach to developing models that predict fat content.

Time-domain nuclear magnetic resonance (TD-NMR) was recently demonstrated to be a promising non-invasive technique for measuring fat content through packaging—with almost no limitations on sample size, and without weighing the samples. Products can be measured one-by-one, promoting accurate quality control for final consumers [1].

TD-NMR spectrometers have been used to measure packaged mayonnaise, salad dressing, nuts, and raw beef. In this article, we will show some results that describe the use of the TD-NMR signals profile and the chemometrics approach to developing models that predict fat content.

USING THE TD-NMR SIGNAL

The NMR phenomenon is observed when a nucleus with an intrinsic angular momentum, or spin, and an associated magnetic moment are placed in an external magnetic field. For samples containing nuclei with spin $\frac{1}{2}$, such as ^1H , ^{19}F , the magnetic field separated the spins into two energy levels. The TD-NMR signal is observed when a sample is irradiated with electromagnetic radiation in the radio frequency region (between 2 and 80 MHz) that matches the energy difference between the two spin levels. After the irradiation, the NMR signal decays as a function of time; this is known as free induction decay, or FID. The NMR signal can also be manipulated by several pulses to measure several NMR parameters, such as the longitudinal (T_1) and transverse (T_2) relaxation times. The sequence developed by Carr-Purcell and Meiboom-Gill (CPMG) to measure T_2 has been widely used to measure fat content in food [2].

The determination of fat content is based on the fact that transverse relaxation time (T_2) of ^1H depends on the effect of molecular motion in the homonuclear dipolar interaction. In the case of fats and other macromolecules, T_2 is known to correlate, in macroscopic materials, with such properties as viscosity, melting temperature, and crystallinity. Thus, T_2 obtained with CPMG pulse sequence can be used for the non-invasive characterization of fat content and fat quality in food products.

DEVELOPING CHEMOMETRIC MODELS

Our research today aims to evaluate this type of signal using chemometric models [3]. The main goal is to predict more than one parameter using a single model, since water and fat content are linked to several properties of food.

Developing such models generally involves an exploratory analysis of the original data to verify the correlation between the variables and their influence in the data set—and, additionally, to assess the tendency of clustering among samples. Principal component analysis (PCA) is the most disseminated chemometric tool used to exploit several features as shown in Fig. 1. The regression method partial least squares (PLS) is based on PCA. The algorithm used to calculate PLS is the Nonlinear Iterative Partial Least Squares (NIPALS).

When using PLS, it is necessary to choose a group of samples which comprise a training set. With this set, a calibration model is built to relate the instrumental response or independent variables (matrix **X**) with the property of interest or dependent variable (**Y**, vector or matrix) of a given sample type by means of a vector (**b**) containing the regression coefficients. The instrumental responses (**X**) will be the TD-NMR signals. The properties of the samples (examples, fat content, moisture and other parameters) are the **Y** matrix.

For PLS, two decompositions are calculated by PCA. The first one to matrix **X**, and the second one to **Y**. The main goal of the PLS is to find a correlation between **X** and **Y**. An internal relationship can be achieved by observing the scores of **Y** (**u**) and **X** (**t**) by a linear relationship according to Equation 1, in which b_h are the regression coefficients.

$$\hat{U}_h = b_h t_h$$

Equation 1

The regression coefficients may be used to predict different properties, such as the fat content of meat.

Another aspect to be pointed out about PLS is that an external set of samples must be used in addition to the training set. These external samples are not used to construct the model, but are instead used to validate the suggested model. When choosing the number of latent variables that will be used in the PLS, it is important to remember that: 1. the variance explained by each latent variable, and 2. the predictive residual error sum of squares (PRESS). The appropriate number of latent variables is one in which the value of PRESS is low. Other values to be observed are the root mean square errors of calibration (RMSEC) and validation (RMSEV).

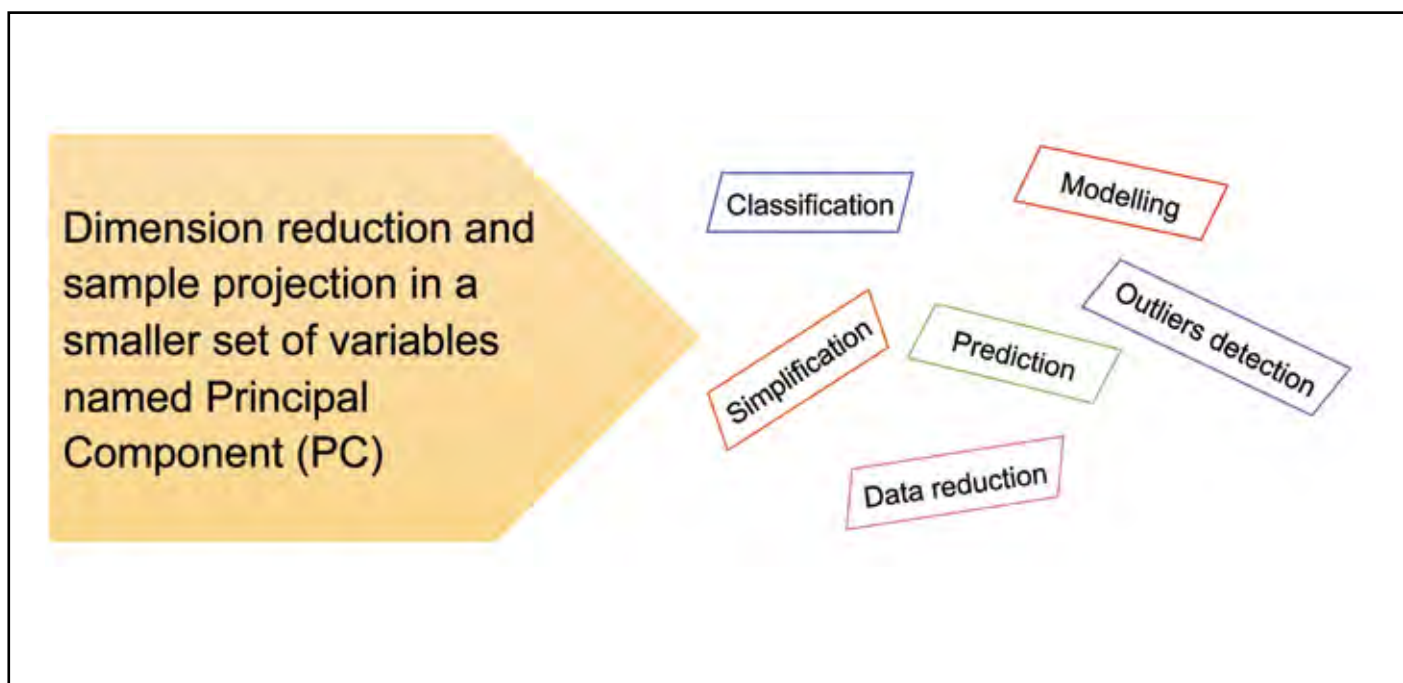


FIG 1: Principal component analysis.

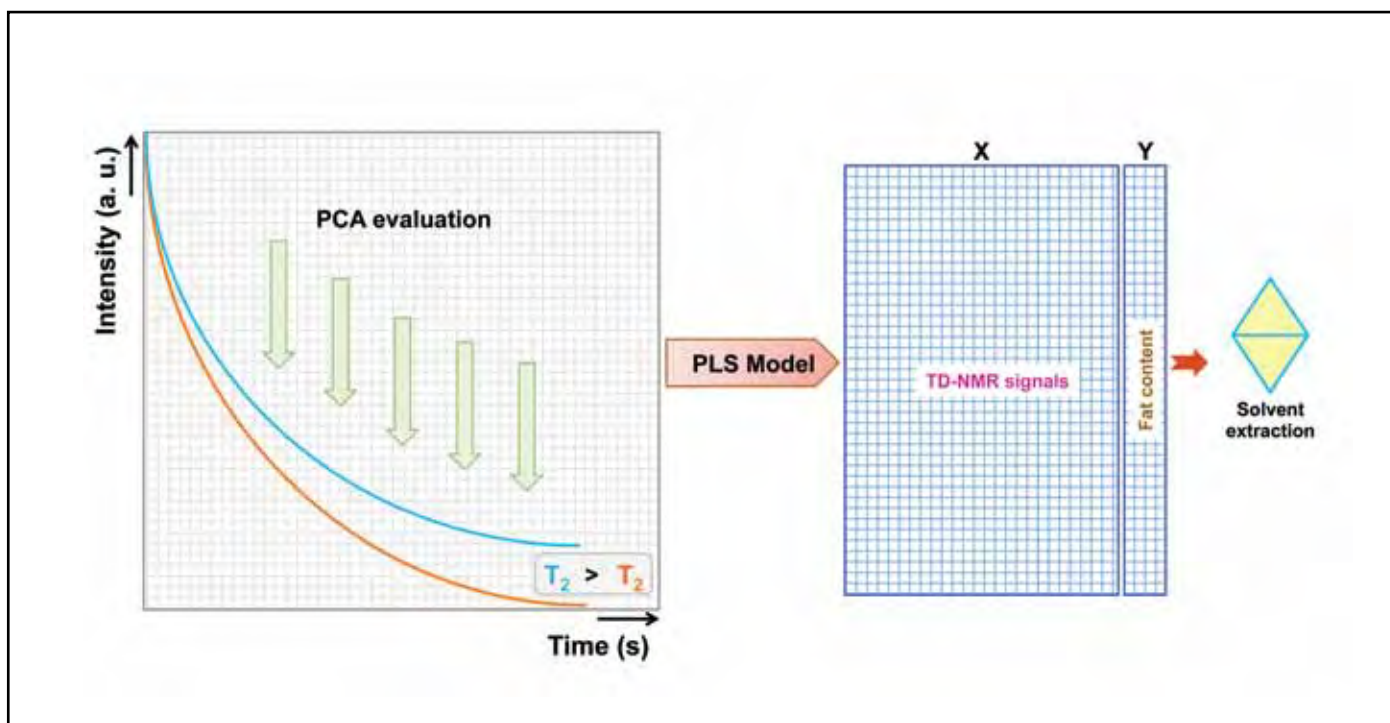


FIG. 2. TD-NMR signals evaluated with chemometric tools

Fig. 2 provides a visual depiction of the differences between the relaxation decays, when the blue line is longer than the orange one. It implies differences in sample composition. Higher values of T_2 are related with less content of fat or more water content, and the opposite is true.

It is standard practice to calculate only T_2 values to determine the fat content, but this information is insufficient for tenue differences between the signals.

With chemometrics tools such as PLS, the relation for each signal is computed based on the reference values obtained from standard methods, and a robust model can be developed. Wet analysis cannot be replaced or fully eliminated, as the multivariate model must occasionally be validated. However, the number of wet analyses requiring solvent extractions may be drastically reduced.

Our research showed that the determination of fat content in emulsion-based foods through the packets used for mayonnaise and dressing salads was possible with a TD-NMR instrument and multivariate models [4].

The permanent Halbach magnet of 0.23 T (9 MHz for ^1H) and a 50-cm-long analytical magnet allowed us to measure the entire package contents [4]. The reference values from Bligh and Dyer's method for fat content were the dependent variable of matrix **Y**, as depicted in Fig. 2. The relaxation decays shown in this figure were comprised with the samples on rows and the instrumental response on columns (time).

The main advantage of the multivariate model over standard methods of wet chemical analysis was better repeatability in determining fat content in emulsions, as repeatability is a key limitation in lipid extraction due to variations in droplet size. Our solution for measuring fat in foods that are intact in their packaging was demonstrated to be feasible. Once each model was developed, few chemical wet analyses need to be

performed. The models described were highly correlated ($r > 0.9$), and correlated between the reference values and the values predicted by PLS.

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Further reading

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Critical design phases for upgrading oilseeds facilities

Matthew Williamson

Before beginning work on a major upgrade or expansion project, you should consider the state of your existing facility, as well as the business justification and long-term site plans. Just as you must harvest a plant before extracting the oil, you must ensure that your facility is fully prepared for an upgrade. Following are some of the items you should consider.

- Upgrading or expanding an oilseeds facility takes a major commitment of time and resources.
- This article summarizes key considerations you should review before beginning such a project.
- It also includes a step-by-step process you can follow, divided into five project phases.

PROPER DOCUMENTATION

Inaccurate, insufficient, or even missing documentation of your existing processes, utilities, and facility infrastructure will lead to rework, which will increase the time and cost for any upgrades or expansion projects. In the area of your facility that will be impacted by the proposed project change, you will need to verify and update four types of information: 1. existing process documentation; 2. site layout drawings and plans; 3. facility civil, structural, and architectural documentation; and 4. design standards. The key documents that you will need for each of these include:

Process documentation

- Process flow diagrams
- Heat and material balances
- Piping and instrumentation diagrams
- Utility balances and available capacities

Site layout drawings and plans

- Plot plan
- Equipment general arrangements
- Electrical area classifications
- Undergrounds map
- Fire protection drawings
- Site master plans

Civil, structural and architectural documentation

- Topographical survey
- Project area geotechnical soil reports
- Structural foundation drawings
- Structural support steel drawings
- Building architectural drawings

Design standards

- Drawing standards
- Piping standards
- Electrical standards

HEALTH, SAFETY, SECURITY, AND ENVIRONMENTAL FACTORS

Any change to your facility will require you to determine your permit needs so that you can evaluate the impact your project might have on your emissions limits and environmental control devices. If you are making any changes to a hexane extraction process, you will have to update your process safety management documentation and implement management of change procedures to gain approval from site safety personnel. Even outside of extraction facilities, most oilseeds facilities with preparation sections must consider the potential impacts of the project on combustible dust safety. Other items to consider include updates to your storm water pollution prevention plan and site security.

FOOD SAFETY REGULATIONS

Food safety regulations are becoming ever more stringent around the world. In the United States, for example, facilities must be sure to maintain the appropriate protocol and documentation to stay current in accordance with plans like Hazard Analysis & Critical Control Points (HACCP) and to comply with the new requirements of the Food Safety Modernization Act (FSMA). Your facility must determine where to draw the boundaries of clean design by implementing post-kill zones, cleaning standards, sanitary designs for piping and infrastructure, and standards for the finished oil blending, loadout, and packing areas.

CONTROL SYSTEMS ARCHITECTURE

As control systems technology continues to evolve, you need to think about the functionality of your current process control platform and how it will be upgraded to support your new project. Many systems in older facilities are no longer supported and cannot be simply upgraded. They may need to be replaced, resulting in major cost and effort for the site. You must also weigh the benefits and risks of remote data acquisition such as Supervisory Control and Data Acquisition (SCADA) to ensure the protection of your data and intellectual property.

PROJECT PHASES

Phase one: feasibility

The initial step of any capital project begins with the feasibility phase, which starts with scaling up any trials completed by Research & Development (R&D). The purpose of this phase is to develop options and analyze the technical and economic

viability of the project. Process engineers, in conjunction with R&D data and support, will develop multiple feasible process options to produce the desired product or achieve the desired process upgrade, such as the addition of water degumming to produce lecithin at an existing soybean oil refinery. Choices made during this phase generally have the greatest impact on the economic and technical success of the project. Block flow diagrams can help you begin to plan the physical flow of your facility changes, while a *pro forma* economic analysis can establish your potential operating costs and project payback. However, due to the number of choices still to be made and the remaining technical uncertainty, capital cost estimates can be no more accurate than $\pm 50\%$ in this phase. This estimate is useful for making critical choices, such as whether or not a project is worth pursuing in greater detail and which key technology choice is best, but it is not sufficient for appropriation funding and should not be relied upon.

Phase two: conceptual

At this stage, you should begin to narrow your options and define your production volumes and impacts, as well as determine the necessary design requirements. The lead process engineer will also begin to outline key process options that mitigate technical, cost and scheduling risks. Available new technologies, such as three-dimensional (3D) laser scanning, will help identify the facility's base conditions and space constraints for planned equipment installation. This is especially helpful for older plants with poor documentation of their existing layout and for heavily congested process areas. During the conceptual phase, you can develop process flow diagrams, a milestone schedule to plan purchasing and construction, and refine cost estimates to $\pm 30\%$. To complete this phase, you need to fully document your existing site conditions with all of the key considerations already described.

Phase three: definition

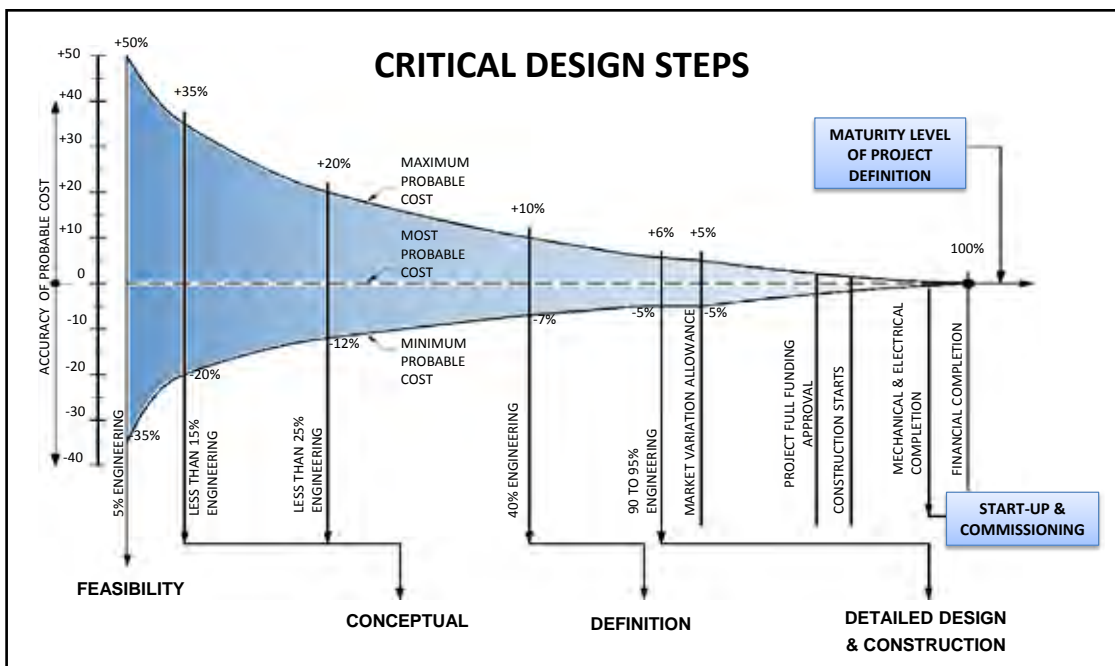
By the end of phase three, all of your front-end engineering (preliminary) design work will be completed, allowing you to select final technical options and finalize your piping and instrumentation diagrams (P&IDs) and general arrangements. Construction bid packages will be analyzed, allowing you to estimate your capital costs within $\pm 10\%$ for capital appropriations. To ensure the desired completion date is met, now is the time to order any equipment that has a long lead time and to secure the appropriate permits for construction. To complete the definition phase, you need to fully document the process design for detailed engineering to begin. Process changes beyond this point may result in significant rework costs and schedule delays.

Phase four: detailed design

During the detailed design phase, all of the detailed information necessary for construction, purchasing and start-up will be completed. In this timeframe, all of the mechanical layouts, piping, structural, and electrical design will be finalized and control programming will be developed.

Phase five: construction and start-up

In the final phase, any last bid evaluations and selections will be made, and you will start to monitor construction administration and safety protocols. You can use construction punch lists to help document changes and modifications along the way, as well as keep track of any missed steps. Start-up begins with commissioning, during which static and dynamic equipment checks and hydro-tests will be performed to demonstrate that the equipment and instrumentation has been installed and is functioning according to design. This phase also includes steps for validation, when the entire process will be tested to prove its ability to meet the project success criteria for operation.



The critical design steps for capital projects can be divided into five phases: feasibility, conceptual, definition, detailed design, and construction and start-up.

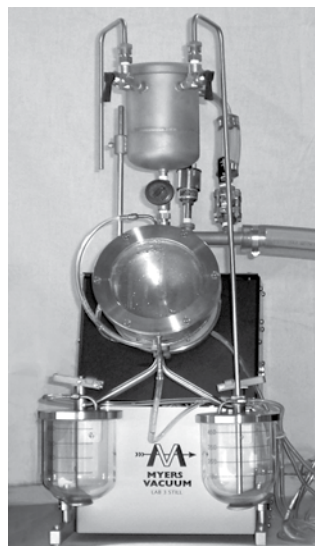
The critical design steps for capital projects can be divided into five phases: feasibility, conceptual, definition, detailed design, and construction and start-up.

Continuous Improvement

Once start-up is complete and the project has been validated, project learning and "as built" drawings need to be documented and filed for future work. Continuous improvement describes efforts to improve reliability and drive down costs outside of structured project activity. This effort is led by site operations or maintenance teams, who will often involve outside engineering resources to evaluate their operational costs and make recommendations for future improvements. Additional documentation may be developed, including an overall project assessment report, an updated site master plan, an energy usage analysis, utility and water balances, reliability assessments, process control loop health measurement, and process and operational loss charts.

While any major upgrade or expansion can feel overwhelming, it is important to visualize the project in manageable steps. Choices made early in the process have a profound impact on the work and the success or failure of the project. It is also critical to understand that an accurate capital cost estimate, as needed for capital project funding, cannot be achieved without completing preliminary or front-end engineering design. This may mean seeking pre-project funding in order for engineering to later obtain full project funding, depending on the complexity of the project. A clear and consistent approach to the engineering work process will ensure the greatest chance of project success.

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The flaxseed portfolio of natural health

Martin J.T. Reaney and Youn Young Shim

ingredients

Lignans, omega-3 (ω -3) fatty acids, orbitides, and high-molecular-weight polysaccharides are flaxseed (*Linum usitatissimum* L.) constituents that are associated with positive health outcomes. Unfortunately, flaxseed also contains bioactive compounds such as cyanogenic glycosides, cadmium, and linatine that are associated with undesired health effects (Fig. 1). Consumption of flaxseed, flaxseed oil, or partially defatted flaxseed meal can potentially deliver a wide range of beneficial health effects (1). For this reason, research conducted to determine the health effects of flaxseed oil, flaxseed meal, and milled flaxseed usually includes studies of two or more functional constituents. Flaxseed and flaxseed meal both contain the complete portfolio, albeit at different ratios. Flaxseed oil and gum products contain two or more bioactive substances. Only highly enriched lignan and orbitide products are sources of a sole constituent of the portfolio.

This article:

- examines the prospects for expanding flaxseed production and consumption as a source of ω -3 oils;
- summarizes the nutraceutical and functional aspects of flaxseed products;
- highlights several new commercial flaxseed products.

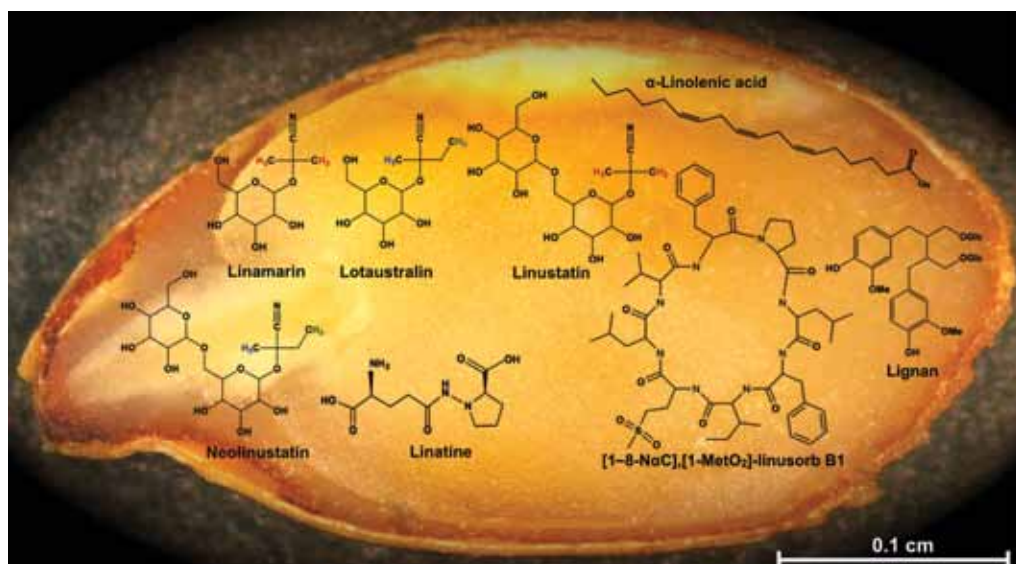


FIG. 1. Structures of four cyanogenic glycosides (mono-glycosides: linamarin, lotaustralin; di-glycosides: linustatin and neolinustatin), linatine, [1-8-NaC], [1-MetO2]-linusorb B1, lignan, linatine, and α -linolenic acid in flaxseed

COMMODITIES WITH POTENTIALLY NEW AND EXPANDING MARKETS

The relatively high cost of flaxseed boutique oils is not surprising but flaxseed oil can be a much less expensive product. Oil crushed in small facilities is, of necessity, expensive. Flaxseed grown in Canada has sold at a small to medium premium (<20%) over canola for the last three years (AAFC weekly price report) even though flaxseed oil content is similar to that of canola. Flaxseed is a commodity, and oil from flaxseed can be produced at costs that are comparable to canola oil if crushing margins are similar. In addition, it is possible to produce considerably more flaxseed than is currently grown, as cultivation practices are improving as are margins for farmers. Flaxseed has lower yields than competing crops but is correspondingly less expensive to grow.

Two flaxseed cultivars (VT50® and HiOmega®) have the highest linolenic acid levels available in a food crop (>68%). There is no yield penalty for growing VT50® when comparing production with other flaxseed varieties. With significant demand, linolenic content of flaxseed could be raised to 68% or more.

The competitive cost of flaxseed and flaxseed oil might lead to a range of bulk products that would be sold in growing volumes. For example, flaxseed oil can be fed to trout and poultry and effectively converted *in vivo* to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), but care must be taken in oil preservation as oxidation leads to poor α -linolenic acid (ALA) conversion to DHA. Archer Daniels Midland (ADM, Red Wing, Minnesota, USA) sells flaxseed oil with added antioxidants that

limit oxidation and note 11 h stability of flaxseed oil treated with 200 ppm TBHQ (2). Fish fed a blend of flaxseed oil with coriander oil (petroselinic acid) showed more efficient conversion of ALA to EPA and DHA than fish fed flaxseed oil alone. This finding might be exploited to achieve superior compositions of animal fats produced in animals fed flaxseed. Global demand for sources of ω -3 oils cannot be met by exploitation of fish stocks, but inclusion of flaxseed or its oil with other oils and foods is a simple approach to increase the ratio of ω -3 to ω -6 in diets and tissues. Adding flaxseed products with the highest ALA content is the most effective way to improve ω -3/ ω -6 ratios. Flax-based linolenic-acid-rich oil products might be included in feed and food with antioxidant additives and lipids that enhance ALA conversion to EPA and DHA. Global demand for feeds with enhanced ω -3 content, vegetable oil sources that can effectively balance ω -3/ ω -6 ratios, are large and growing.

NOT ALL FLAXSEED IS EQUAL

Health Canada has approved claims on product labels that relate flaxseed consumption with reduction of blood cholesterol levels and research has also shown that consumption of milled flaxseed lowers blood pressure in hypertensive patients (3). Effects of flaxseed consumption on health might arise from ALA, high molecular weight polysaccharides and lignan but contributions from other bioactive compounds are likely. Biologically active orbitides, for example, might contribute to flaxseed effects on both blood pressure and cholesterol.

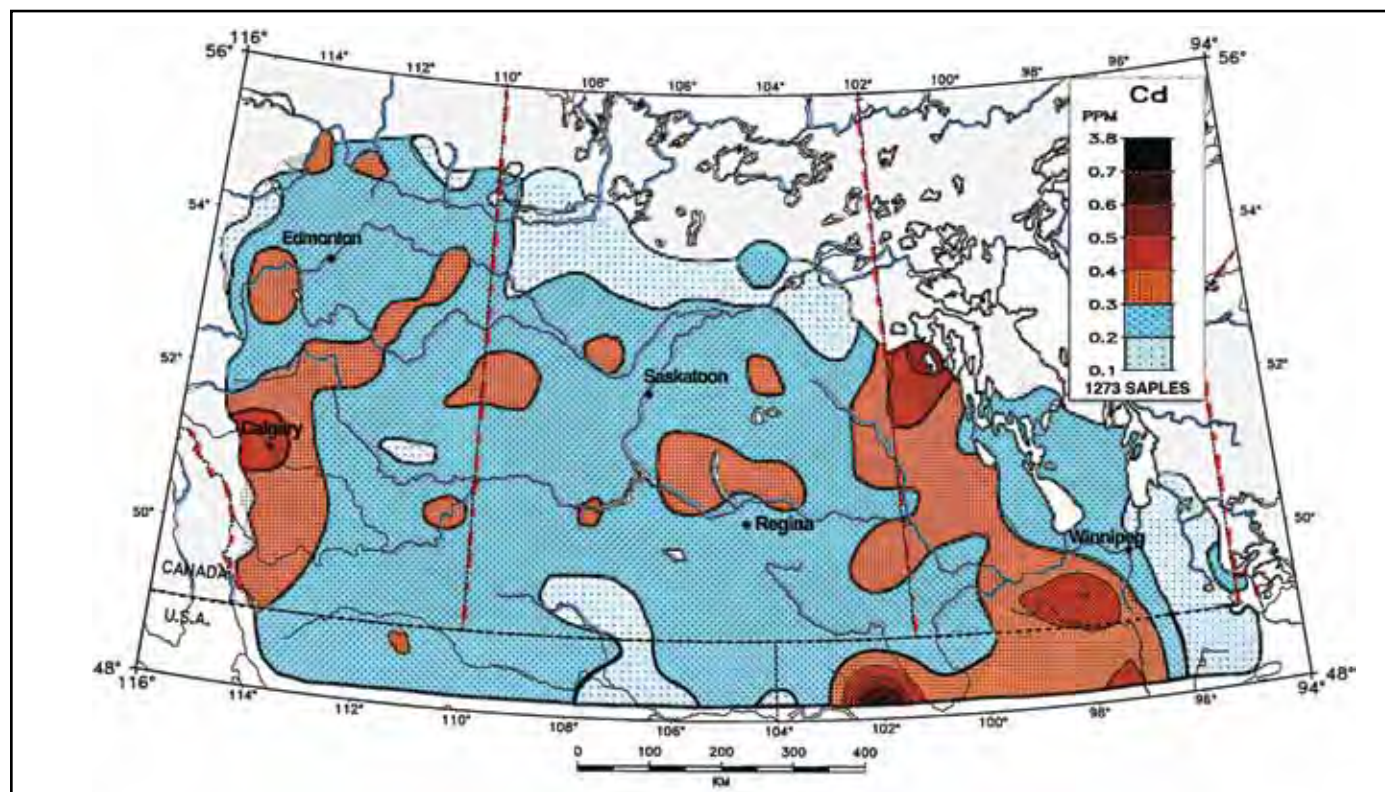


FIG. 2. Regional distribution of cadmium in prairie Ap horizon soils

Flaxseed bioactive constituents have never been standardized through breeding. Therefore, it is possible that more potent and less potent flaxseed cultivars might be available. Most studies of the effects of flaxseed and flaxseed products on health do not fully document the flaxseed source or the total portfolio of active ingredients. Flaxseed, its oil, and its meal may be incorporated into food with minimal processing. Flaxseed is sold whole or as a milled product, and it is added directly to foods. Consumption of milled flaxseed is generally recognized as safe (GRN No. 280, 4). Flaxseed takes up cadmium from the soil but not all soils have the same cadmium content. For example, flaxseed grows in areas of northern Canada, where soils have little cadmium and produce seed and meal with lower cadmium content (Fig. 2, page 23).

IMPROVING AGRONOMIC TRAITS, OIL CONTENT, AND ALA

Flaxseed production is remarkably small compared with canola and soybean. Specifications of canola cultivars are comparatively more rigid than for flaxseed cultivars. Canola cultivars must achieve high yields, be resistant to common diseases and stress, and must meet minimum specifications for low-saturate, erucic acid, and glucosinolate content. The composition requirements for defining new flaxseed cultivars are less regimented. Variability in flaxseed cultivar quality could make it difficult to reliably source flaxseed with known contents of lignan, dietary fiber, and

orbitide or, for that matter, cyanogenic glycosides, cadmium, and linatine. Future flaxseed cultivars will likely have defined content of several of these constituents. Flaxseed cultivars will be tested to determine if they impart expected health-promoting effects.

FLAXSEED PRODUCT COMPOSITION

Flaxseed and flaxseed meal have the highest levels of lignan of any known food. Sesame seed has one-tenth the lignan found in flaxseed. Flaxseed lignan extracts contain over 50% lignan while high lignan oil products contain flaxseed particulate (~10%). Such products contain just 0.1 to 0.2% lignan. It is uncertain that there is sufficient lignan in a daily dose of high lignan oil to have any health impact. The level of orbitide and other cyclic peptides in foods is poorly studied. Flaxseed oil has the highest known orbitide concentration of any food. Flaxseed orbitides induce anti-inflammatory responses in cell cultures but they are poorly absorbed. It is not known if these compounds are absorbed when flaxseed or its products are consumed.

Two recent patent filings point to possible novel applications. Orbitides fed to poultry at 0.2% of diet decreased intestinal inflammation. Another patent application identifies [1–8-NQ₂C], [1-MetO₂]-linusorb B1 (a.k.a. cyclolinopeptide J) as a drug lead for treating osteoporosis and a host of other bone loss conditions. Ten-nanomolar orbitide caused a 50% inhibition of osteoclast cell division. Flaxseed meal is being used as a functional ingredient in gluten free goods as a replacement for thickeners such as xanthan gum. Recently, we have shown that flaxseed

TABLE 1. Commercial products of flaxseed meal or hull extracts

Product name	Active ingredient	Manufacture	Benefit
BeneFlax™	SDG, vitamin D, whey powder	Archer Daniels Midland Company	Decreasing oxidative stress and inflammation should improve a number of the problems associated with aging
LignaMax™	A stabilized lignan	Bioriginal Food & Science Corp.	Non-GMO, 4 times more of the lignan SDG than regular milled flaxseed
LinumLife®	Rich in lignans	Frutarom	“Balancing act” in the human body by modulating the circulating hormone levels and providing an antioxidant effect
AlaLife® Flax Lignans	High quality of lignans-SDG	BioGin Biochemicals Co., Ltd.	For women: weight management, benefit for breast health, enhanced effect combined with isoflavone, effect on lipid and bone/ amendment effect of fever; For men: prostate health/hair loss
Sensiline®	Hydrolyzed linseed Extract	Silab	An innovative active ingredient for sensitive skin: Soothing
Flaxseed-Orbitide®	Rich in orbitides	Prairie Tide Chemicals Inc.	Antioxidant and anti-inflammatory

stabilizes mixtures of gums with protein and actually is a naturally occurring coacervate with conlinin protein. As such flaxseed gum might see increasing utility as a food ingredient. A series of commercial products produced by extraction of flaxseed, flaxseed meal or flaxseed hulls are presented in Table 1.

FLAXSEED PRODUCT TRENDS

Flaxseed consumption is increasing and there is a great opportunity for expanded production to match increased demand. New lower cost products will see increased use of flaxseed oil, and possibly meal products in feed and food. Flaxseed cultivars with proven and improved composition will be used for health products to maintain or improve its value to consumers. Moreover, new highly enriched products are emerging from the flaxseed portfolio that provide specific benefits to consumers. Flaxseed consumer sophistication will align with equally sophisticated new products.

Dr. Martin J. T. Reaney is a professor in the College of Agriculture and Bioresources at the University of Saskatchewan, and is also the Saskatchewan Ministry of Agriculture (SMA) Chair of Lipid Quality and Utilization. The SMA chair is mandated to develop new technology for oilseed processing and producing commercial bioproducts with enhanced value. The commercial activity resulting from this research is intended to generate wealth for the Canadian agriculture sector. Reaney's research interest involves exploring orbitides compounds including a range of natural health products, pharmaceuticals, and cosmetics, and developing technology to bring these compounds to the market for a broad range of applications. He recently formed Prairie Tide Chemicals Inc. (PTC) to commercialize several recent discoveries of an abundant source of flax compounds. He can be contacted at martin.reaney@usask.ca.

Dr. Youn Young Shim is a Research Officer of Lipid Quality and Utilization in the Department of Plant Sciences at the University of Saskatchewan and a Chief Science Officer in Prairie Tide Chemicals Inc. Her current research interest is to determine the role of flaxseed proteins and peptides in health outcomes. Shim coordinates research activities with partner agencies and collaborators in Europe and Asia.

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Multi-spectral hand-held devices for edible oil and general food inspection

Tremendous advances in integrated electronics and sensors make it possible to design extremely powerful in-line measurement systems that provide quasi real-time feedback in industrial processes. Such systems are widely used in the food industry to monitor food quality.

- Sharp increases in the number and diversity of fraudulent products and food adulteration, together with the public's growing interest in the freshness and nutritional contents of foods, have led to the development of hand-held devices consumers can use to sample and analyze foods at home or in restaurants.
- Assisted living has created an additional need for food preparation and culinary assistance systems that help residents with sensory degradation or impairment detect spoiled, rancid, poorly prepared, or nutritionally deficient food.
- This article describes how autonomous, multi-sensory, wireless measurement systems incorporated into household items of daily use can be used to distinguish a range of ingredients from one another and from adulterated samples.

Andreas König and Kittikhun Thongpull

Unfortunately, the retail-to-fork portion of the food chain has become increasingly vulnerable to the insertion of fraudulent, adulterated, misrepresented, and mislabeled food products. In January 2016, Italian police confiscated 7,000 tons of olive oil from North Africa that had been deodorized with chemicals and rebranded as more expensive Italian extra virgin olive oil (<http://www.cbsnews.com/news/60-minutes-agro-mafia-food-fraud/>). And that is just olive oil. In a two-month crackdown that occurred a year earlier, the International Criminal Police Organization (Interpol) seized 2,500 tons of fake or adulterated food and 275,000 liters of tainted drinks from markets, airports, seaports, and shops in 47 countries. Those confiscated products included cooking oil, fake butter, cheese, meat, seafood, produce, bottled mineral water, and alcohol. One plant in the United Kingdom was caught making fraudulent brand-name vodka in antifreeze containers and treating it to remove the chemical smell (<http://time.com/3711938/food-fraud-interpol/>)!

In response to consumer concerns about food fraud and the public's growing interest in the freshness and nutritional contents of the foods they eat, several competing hand-held systems designed for quick and easy sampling and analysis of food (such as the Food-Sniffer, the Scio, and the Telspec systems, among others) have entered the consumer market.

The field of living assistance has provided an additional incentive to develop food preparation or culinary assistance systems, as the daily life activities of food preparation can be challenging for many consumers due to restricted skills, experience, and accident- or aging-induced degradation or loss of perceptive abilities.

Consequently, in our research work [1], we have conceived the E-Taster-assistance-system for living assistance (Ambient-Assisted-Living, AAL) and food safety. Autonomous, multi-sensory, wireless measurement systems (Lab-on-Spoon/-Fork, LoX), which have been embodied as items of daily use, provide an “electronic tongues” sensory context for the required assistance at the consumer end of the food chain. The E-Taster system includes an electronic cookbook that interacts with the multi-spectral LoX devices and also has special functions for sustainable use of food. The LoX devices can be understood as hybrids between AAL and Lab-on-Chip activities, focusing on the needs of a cost-sensitive mass-market.

MULTI-SPECTRAL MEASUREMENT

The current version of the Lab-on-Spoon employs impedance spectroscopy and visual and near infrared range spectroscopy. Currently, the impedance measurement is based on an AD5933 chip working in a range only up to 100 kHz. Even this moderate range is already allowing the discernment of a rich range of ingredients from each other and, in particular, from adulterated samples. Two gold-plated electrodes are placed in the cavity of the 3D-printed spoon package (Fig. 1).

The visual and near infrared range spectroscopy is achieved by a MAZet MMCS6CS multicolor sensor with two MCDC04 readout chips and appropriate active illumination. Currently, we use a white light LED and a 750 nm LED and extract 16 spectral values in two measurement cycles. The most recent version has been extended to 850 nm and 355 nm illumination. In particular, for oil and lubricants assessment, such as in combustion engines or gear boxes, infrared spectroscopy has established itself as a powerful means, and the LoX implements a low-cost realization of it. The UV-LED extension opens the door to fluorescence-based analysis and increased discriminance, at a feasible cost. For calibration and food preparation reasons, the temperature of the spoon contents is monitored, employing a UST Pt10k sensor and readout circuit. Fig. 2 (page 28) shows the most recent version of the MS-LoS with 850/355 nm extension. Overall, we extract a fingerprint consisting of one temperature value, 16 (32) visual/NIR range values, and 1,024 impedance-related magnitude and phase values.

The LoX are conceived as 3.3 V systems, like many comparable Internet of Things (IoT) devices, based on an Arduino Pro Mini (3.3V, 8 MHz) micro-controller. The autonomous MS-LoS includes an Li-Po-accumulator, that will be charged via the USB connector. Communication with the server and the E-Taster-assistance-system is achieved either by USB or wireless by the XBee interface. More details of the technical solution and application are covered in our video [2]. The Lab-on-Fork device has been added to the family to enable



FIG.1. Multi-spectral Lab-on-Spoon with NIR and UV extension for fluorescence based analysis

the inspection of a wider spectrum of food in addition to liquids, such as solid foods (fish or meat, for example), granular food, or powders [2]. The LoX devices extract fingerprints of food samples based on their measurement principles, which are then processed by the algorithms outlined in the next section of this article. However, it is essential that these cost-sensitively acquired fingerprints be correlated with the “ground truth,” such as from a priori knowledge or profound laboratory analysis.

ANOMALY OR NOVELTY DETECTION

In our E-Taster-assistance-system, the extracted fingerprints of the spoon contents are communicated to the host and E-Taster application with, for example, the E-cookbook. There are now two ways to go in further food assessment. In the first approach, the system would try by multi-class classification to determine the spoon’s contents based on a database of correlated ingredients/fingerprints. The more ingredients or classes are in the system, the harder and more error-prone decision making becomes. Further, mandatory extension of the database requires corresponding retraining of the decision-making units.

The second approach seems to be more promising in numerous applications, as it is based on anomaly or novelty detection, also denoted as one-class classification, such as that used by our NOVCLASS method. In OCC, the decision making unit is trained with a set of characteristic fingerprints from the expected ingredient and it will discern deviations from this representation without even the need for counter examples. This discloses product deviations as well as

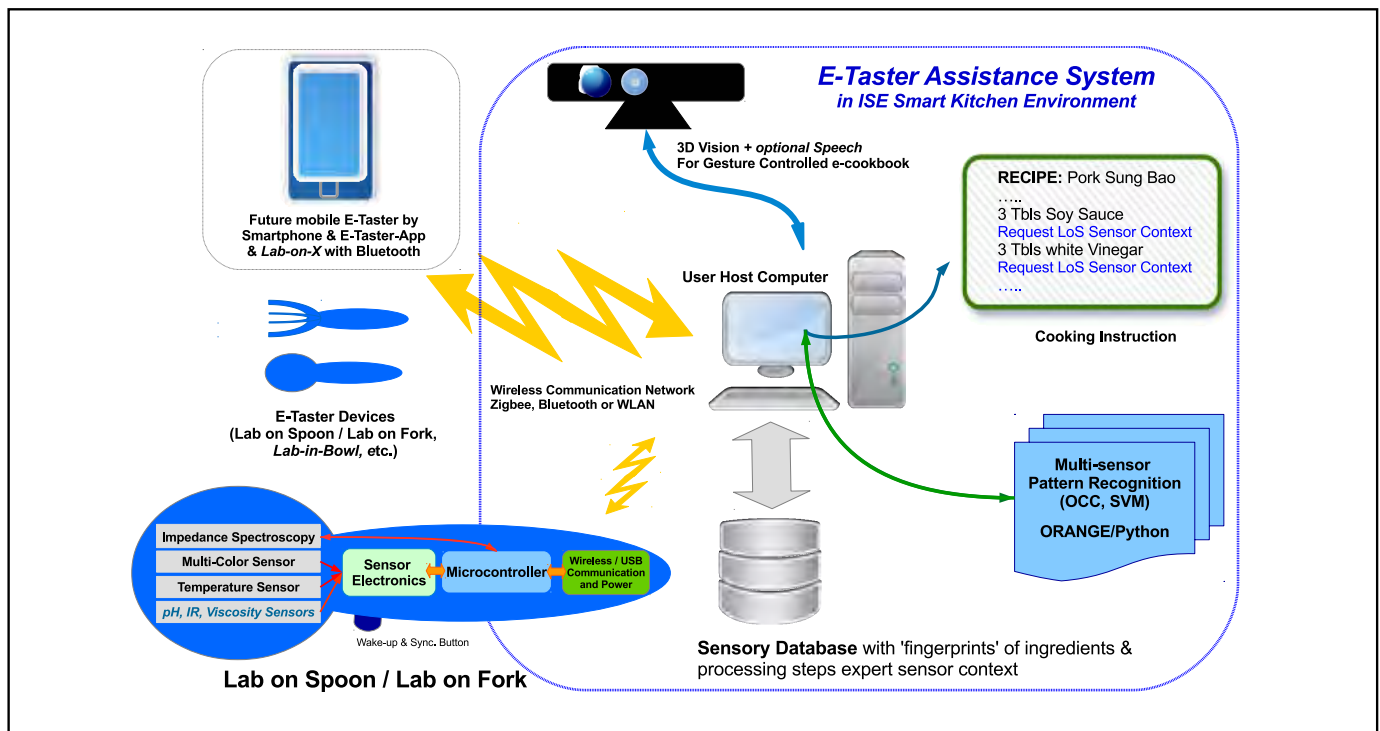


FIG. 2. Block diagram of the E-Taster-assistance-system with LoX devices and mobile extension

fraudulent products with a high sensitivity and minimum training effort. The E-cookbook calls for each expected ingredient in a recipe step on the corresponding decision-making unit. New ingredients and corresponding decision-making units can easily be added to the database. Even an identification of an unknown ingredient can be approached by sequentially calling on the OCC units in the database and finding an acceptable match [2].

Further, the raw fingerprint will be subject to feature computation and dimensionality reduction to remove redundancy and irrelevance for the specific task and obtain a lean yet better discerning decision-making unit. For the rapid and dependable extension and adaptation to increasing tasks, our DAICOX-system for the automated design of optimized technical cognition systems will be employed.

OIL RECOGNITION OR GRADING

The LoX-devices have been used in numerous tasks, such as detecting freshness and decay in milk and contamination in wine; recognizing types of fish or meat; and discerning types, grades, and conditions of various edible oils.

These capabilities are summarized and partially demonstrated in the corresponding video [2]. Using the LoX-devices to identify and assess the type, grade, and condition of edible oils can, in many respects, follow the approach used to assess similar characteristics in the non-edible oils and lubricants used in engines, gearboxes, and windmills. Fig. 3 shows the NIR range spectroscopic data for five different vegetable oils that were examined, based on 15 samples of each.: (1) peanut oil, quality A, (2) soybean oil, (3) olive oil (4) sunflower oil, and (5) peanut oil, quality B. In spite of scatter within each of the oil categories, the different varieties of oil can be clearly distinguished from

one other, and the two qualities of peanut oil are also discernible from the similarity or feature space plot. Thus, a fraudulent oil product could potentially be detected by comparing it to the fingerprint of the oil that is expected to be used in a particular cooking step. On the other hand, analyzing the composition and detailed contents of a fraudulent oil is not within the scope of the current LoX devices.

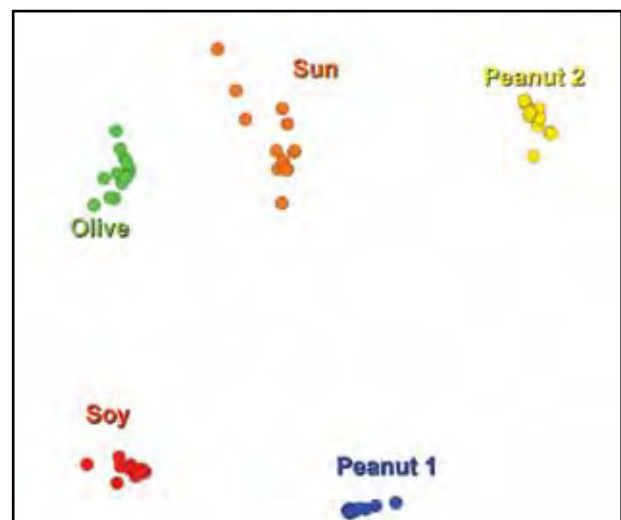


FIG. 3. Plot showing the similarity and discernibility of five edible oil samples based on the selection of channel 8 and 28 of the visual and NIR range spectroscopic data, clockwise from 5 o'clock: quality A peanut oil (purple), soybean oil (red), olive oil (green), sunflower oil (orange), and quality B peanut oil (yellow).

MORE RESEARCH AND IMPROVEMENTS

Our current E-Taster system and ingredient/food fingerprint database is still in a prototypic state, but it has already demonstrated relevant screening capabilities that are suitable for marketing at the consumer end of the food chain. To advance towards a more able measurement system and viable product, numerous improvements are currently being evaluated in version 3 of the LoX devices. Extending the impedance spectroscopy bandwidth to at least 12 MHz is currently being investigated, based on preliminary network analyzer measurements. Further spectral extensions (to a deeper UV of 255 nm, for example, and the inclusion of low-cost viscosity and pH sensing) are being pursued, and we are also working on the migration of the E-Taster system to an app on mobile phones and a metal 3D print of the device package in stainless steel.



Andreas König heads the chair of Integrated Sensory Systems (ISE) at Technische Universität (TU) Kaiserslautern, Germany, where he teaches the foundations of measurement and instrumentation as well as of electronic devices and circuits. His research interests and activities are in the fields of

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Kittikhun Thongpull is a research assistant and PhD candidate at the chair of Integrated Sensory Systems (ISE) of TU Kaiserslautern, Germany. His research interests are in the field of computational intelligence and integrated intelligent sensory systems with self--properties. His PhD thesis focuses on the task of automated design of intelligent multi-sensory systems.*

The authors gratefully acknowledge the project's sponsor, MAZeT, <http://www.mazet.de/en/mazet#.VpZ3YfkrJhF>.

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Top-grade oil

Jay Whetter

Green seed, heated seed, moisture, and dockage all add costs to canola processing, which is why seed delivered with high amounts of any of these factors will fetch a lower price. The bottom line is that top-grade canola with low dockage moves through crushing plants more efficiently.

- **Top-grade canola is worth more for a reason.**
- **Seed quality affects processing costs and may even lower the price one can get for the refined products.**
- **This article describes the steps used to process canola and how seed quality affects them as well as the final quality of the refined oil and meal pellets.**

GREEN SEED

Green canola seed usually has issues other than just high chlorophyll levels. Canola delivered with high levels of green seed was probably swathed immature or was frozen before it had a chance to fully cure. Therefore canola with high green seed content also tends to have smaller seeds, more damaged seeds and a lower overall oil content per ton of seed delivered.

High amounts of green chlorophyll in the seed also increase the processing cost because that chlorophyll must be removed to produce the light-colored oil customers expect.

Processors use a clay filtration process to remove chlorophyll. Natural clay particles—"Fuller's earth" or "montmorillonite clay" used specifically for their high adsorption qualities—are added to the oil. Interestingly, these same clays are used to clarify wine. Chlorophyll molecules bond with the clay particles. The clay is then filtered, taking the chlorophyll with it. Canola oil with higher chlorophyll content will require more clay and possibly more passes with the clay to remove that chlorophyll, adding to the cost required to clarify the oil. "All canola oil gets the clay treatment, but the more chlorophyll, the more clay and cost required for that step," says Dave Thiessen, edible oils facility manager with Bunge in

Altona, Manitoba, Canada. Adel Ghabour, quality assurance manager with Richardson Oilseed in Lethbridge, Alberta, Canada, adds that the more clay used to remove color, the more oil that is lost in the process. "Oil is trapped within gaps in the clay, and that oil is not recovered unless the clay goes back through the extractor," he says.



Adel Ghabour, quality assurance manager with Richardson Oilseed, explains the key steps in canola processing at CanoLAB in Olds, Alberta, Canada.

HEATED SEED

Canola seed damaged in storage by heating does a number of things to degrade quality. Heating will burn off oil, so there is less oil per ton of seed. Heating also changes the color of oil and it increases free fatty acid content. For these reasons, processing plants tend to get very picky about how much they'll take.

Processors can handle low levels of heated seed, but it still adds cost. Heating will "set" the natural red color pigments in canola. All canola oil contains some red pigments, which are removed with the same clay process that removes the green chlorophyll molecules. "Red color set in by heating is very difficult to remove," Thiessen says. "Deodorizers can't remove it, and we end up with darker oil in the end."

Heated canola also has higher levels of free fatty acids. Fatty acids are usually in triglyceride bonds—three fatty acid molecules are attached to a central glycerol molecule. Free fatty acids are broken from the glycerol bond, and these "free" fats greatly reduce the stability and shelf-life of oil.

Free fatty acids are found in all canola. Good quality new canola seeds will have about 0.5% of fatty acids in the free form. After a year in storage, oxidation through the aging process will push that up to around 1.0%. Heated canola will have much higher free fatty levels as heat breaks the glycerol bonds. Free fatty acids are removed with sodium hydroxide—or "soda"—in the refining process. The sodium molecule in the soda attaches to the fatty acid molecule to make "soapstock," which is removed from the oil in a centrifuge.

MOISTURE

The optimum moisture content for canola entering a processing facility is between 7 and 7.5%. That is the target moisture for ideal cooking and flaking results. (See step 2 in "The 11-step process" on page 32.) Processing plants adjust canola moisture up or down as needed before cooking.

"This delays product flow, which is an economic factor," Ghabour says.

Optimum moisture levels are also important to limit the risks associated with seed spoilage and potential incubation of harmful bacteria and mold. Artificially increasing moisture levels by adding water to the canola seed prior to delivery has the potential to exacerbate these risks. Although this is an uncommon practice and illegal in the United States, it is important to understand that adulterating the seed with water to increase product weight not only increases costs associated with handling, it also poses a risk on the quality and safety of the downstream products.

DOCKAGE

Processing plants clean the seed prior to processing. Dockage removed in cleaning the canola is typically added back to the process after extraction. Scalpings, which are items screened out of the seed that cannot be reincorporated into the meal, are taken to the land fill.

CONTINUED ON PAGE 32



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CONTINUED FROM PAGE 31



Canola meal leaves the processing facility in pellet form for easy handling.

“High dockage means extra work for the pre-cleaning step, and it can add extra handling costs if dockage exceeds the amount that can be added to meal,” Ghabour says.

Canola processing is an important and necessary step in producing the end products our customers want. The extra costs to process lower grade canola exhibit precisely why Canada has grading standards and why striving for quality has its economic rewards.

Jay Whetter is the editor of Canola Digest magazine, a joint publication of the Alberta Canola Producers Commission, Sask-Canola, the Manitoba Canola Growers Association and the Canola Council of Canada. This article originally ran in Canola Digest and is reprinted with permission.

The 11-step process

Canola goes through 11 major steps as it moves through a processing facility to be made into refined customer-ready oil and meal pellets.

1. Seed is delivered by truck either by individual producers or grain companies. The truck is inspected and sampled, then graded according to Canadian Grain Commission Standards to determine the value to the vender along with the current price for the grower. Seed is segregated as to the type and/or grade and stored until used by the processing plant. Modern processing plants usually just have a week or two of storage capacity.
2. Seed is cooked and then flaked by roller mills to ensure all oil is exposed to as much surface area as possible. Optimum flake thickness is 0.3–0.38 mm. Flakes thinner than 0.2 mm are very fragile while flakes thicker than 0.4 mm result in lower oil yield.
3. A mechanical screw-type expeller squeezes about two-thirds of the oil from the flakes.
4. After expelling, the high-oil expeller cake (meal plus remaining oil) goes through solvent (hexane) extraction to separate the remaining oil. The cake moves through a long wash chamber as hexane is poured over top, dissolving the oil from the meal.
5. Meal is then heated to remove the hexane, which is condensed and used again. Meal is then ground and/or pelleted for sale into the feed market.
6. Oil removed from cake in step 4 is desolventized by heating it under vacuum. Hexane becomes a gas at 60–65°C, so it boils off at a much lower temperature than the oil, making for easy separation. This gas is vacuumed from the heating chamber and condensed back into a liquid for reuse.
7. Oil removed from expellers in step 2 has the solids removed by filters or centrifuges and is combined with oil from the extraction process in step 6.
8. The combined oil then goes for refining. Step one in refining combines oil with sodium hydroxide—“soda”—to react with water soluble components. This removes gums and free fatty acids to create a product called soapstock, which is a high energy feed ingredient. Soapstock is separated from the oil with a centrifuge.
9. Oil is then clarified using a clay that adsorbs any pigments remaining in the canola oil. Oil is filtered to remove the clay.
10. The final refining step is deodorization. Oil is heated to 240°C under almost perfect vacuum to distill off short chain molecules, such as any remaining free fatty acids, along with flavor and odor compounds. The final product is mild tasting, light colored canola oil.
11. This oil is then weighed and shipped in rail cars, truck tanks or smaller containers to a packaging facility where it is bottled.

“Red colour set in by heating is very difficult to remove. Deodorizers can’t remove it, and we end up with darker oil in the end.” —Dave Thiessen

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Is **fat** really the **sixth taste**?

Laura Cassiday

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

The past year has been full of headlines about the elusive sixth taste: oleogustus, as some researchers have termed it, or “the unique taste of fat” (Running, C. A., *et al.*, <http://dx.doi.org/10.1093/chemse/bjv036>, 2015). Recent studies and review articles have made the case that fat should join salty, sweet, bitter, sour, and umami as one of the basic tastes. But how convincing is the experimental evidence? Is it really time to declare fat the sixth taste?

In a 2015 review article, Russell S. J. Keast and Andrew Costanzo argue that for fat to be considered a taste, it must meet the following criteria: 1) There must be a distinct class of chemicals that stimulate the fat taste; 2) there must be signal transduction mechanisms to convert the chemical stimuli to an electrical signal; 3) the electrical signal must be transmitted by neurons to processing regions of the brain; 4) the fat taste should be distinguishable from other tastes; and 5) there must be physiological effects following activation of the taste bud cells (<http://dx.doi.org/10.1186/2044-7248-4-5>).

Let's examine the evidence for each of these criteria:

1) There must be a distinct class of chemicals that stimulate the fat taste.

Just as the breakdown products of carbohydrates (sugars) and proteins (amino acids) are responsible for sweet and umami tastes, respectively, the likely stimulus for fat taste is fatty acids, the breakdown products of triglycerides. A lipase enzyme in the saliva, called lingual lipase, cleaves a portion of ingested triglycerides into free fatty acids (the remainder are broken down further in the gastrointestinal tract). The released free fatty acids can then bind to putative receptors on taste bud cells to elicit downstream effects.

Humans can easily detect the unpleasant taste of oxidized fatty acids, which are typically present at high concentrations in spoiled food. However, the levels of fatty acids that stimulate fat taste are low enough to not be considered unpleasant, but high enough to activate receptors on taste bud cells (Keast, R. S. J., and Costanzo, A., <http://dx.doi.org/10.1186/2044-7248-4-5>, 2015). In human taste tests, this level corresponded to 0.1%–3% w/v fatty acids inherent in fresh and processed foods (not considering the free fatty acids potentially released by lingual lipase).

2) There must be signal transduction mechanisms to convert the chemical stimuli to an electrical signal.

The most convincing fat taste receptor identified so far is the fatty acid transporter CD36, found on human taste buds cells. Researchers have found that variations in the *CD36* gene

influence fat sensitivity (Pepino, M. Y., *et al.*, <http://dx.doi.org/10.1194/jlr.M021873>, 2012). In a 2012 study, participants were given three different cups of solutions, one of which contained a small amount of a fatty oil. The other two cups contained solutions similar in texture but fat-free. The researchers asked the participants to choose the cup that was different. The same three-cup test was repeated several times, with different concentrations of fat. “If we had asked, ‘does it taste like fat to you?’ that could be very subjective,” said first author M. Yanina Pepino of Washington University School of Medicine in St. Louis, Missouri, USA, in a news release. “So we tried to objectively measure the lowest concentration of fat at which someone could detect a difference.”

People who made the most CD36 were eight times more sensitive to the presence of fat than those who made 50% less of the protein. This study is consistent with animal experiments in which rodents genetically engineered to lack functional *CD36* ceased to display a preference for fatty foods.

The CD36 receptor has an extracellular, hydrophobic pocket that binds both saturated and unsaturated long-chain fatty acids (LCFA) with an affinity in the nanomolar range (Besnard, P., *et al.*, <http://dx.doi.org/10.1152/physrev.00002.2015>, 2016). CD36 and associated LCFA have been detected in lipid rafts—clusters of signaling molecules—in the membranes of taste bud cells. Besnard and colleagues proposed a model (supported by experimental data) in which binding of LCFA to CD36 initiates a signaling cascade in taste bud cells that results in calcium accumulation and cell depolarization, an electrical signal that can be transmitted to the brain. Although the evidence is less consistent, other receptors have been proposed to sense or modulate the fat taste. For example, the G protein-coupled receptor GPR120 on taste bud cells binds to and is activated by fatty acids. However, mice lacking GPR120 still show a preference for fatty foods, indicating that the receptor is not essential for fat taste sensation. It is possible that GPR120 cooperates with CD36 to modulate fat taste sensitivity (Besnard, P., *et al.*, <http://dx.doi.org/10.1152/physrev.00002.2015>, 2016).

3) The electrical signal must be transmitted by neurons to processing regions of the brain.

Upon depolarization, taste bud cells (of which there are four major types with different taste receptors) can release various neurotransmitters such as acetylcholine, serotonin, and norepinephrine (Besnard, P., *et al.*, <http://dx.doi.org/10.1152/physrev.00002.2015>, 2016). These neurotransmitters reach afferent nerve fibers of gustatory neurons that carry the electrical signal to the brain.

Binding of CD36 to LCFA triggers the release of serotonin and norepinephrine from type III taste bud cells. Denervation of the peripheral gustatory nerves in mice suppresses their preference for fats.

4) The fat taste should be distinguishable from other tastes.

The perception of a fatty “taste” was long thought to arise solely from the texture, smell, or appearance of a fatty food. Although a fat taste is not as readily defined as, for example, a sweet or salty taste, a recent study has shown that people can distinguish fat from other tastes in the absence of textural, olfactory, or visual cues (Running, C. A., *et al.*, <http://dx.doi.org/10.1093/chemse/bjv036>, 2015). Researchers gave study participants multiple cups of solutions, each containing a compound corresponding to sweet, salty, bitter, sour, umami, or fatty. They asked the participants to sort the cups into groups of similar “quality or type” (researchers carefully avoided use of the word “taste”). All of the solutions, which were served in opaque cups with lids, had similar viscosities, and participants wore nose clips to keep them from smelling the liquids.

The participants could easily separate sweet, salty, and sour stimuli. They consistently grouped short-chain fatty acids with sour stimuli, which could be expected since acetic acid is also a short-chain fatty acid. There was a large overlap among bitter compounds, medium-chain fatty acids (MCFA), and LCFA, possibly because all were considered unpleasant stimuli. There was also some overlap between MCFA and umami stimuli, but the researchers attributed this result to the participants’ lack of experience with umami sensations, rather than true perceptual overlap. When the participants were given new cups containing only fatty, bitter, and blank compounds, they could clearly distinguish the three groups.

Among fatty acids, LCFA elicited the most unique, perceptible sensations, consistent with the known preference of the CD36 receptor for LCFA.

5) There must be physiological effects following activation of the taste bud cells.

The interactions between fatty acids and specific receptors in taste bud cells have physiological consequences that affect both food intake and digestion (Besnard, P., *et al.*, <http://dx.doi.org/10.1152/physrev.00002.2015>, 2016). The electric signal sent to the brain by the taste buds increases digestive secretion in preparation for the coming food. In addition, the signal stimulates the brain to evaluate the hedonic (pleasure or palatability) value of the food and the metabolic (energy) needs of the organism, which together determine eating behavior. In addition to releasing neurotransmitters, taste bud cells release gastroin-

testinal hormones that regulate digestive tract function, glucose homeostasis, and appetite/satiety.

A brief oral exposure to fats (sipping and spitting, without actually ingesting the fat) causes an immediate rise in plasma triglyceride levels. The physiological significance of this increase is unknown, but it might signal the body to prepare for fat digestion and absorption (Keast, R. S. J., and Costanzo, A., <http://dx.doi.org/10.1186/2044-7248-4-5>, 2015). Other physiological effects of oral fat stimulation include increases in lipase secretion, stimulation of gastrointestinal hormones, and fluctuations in glucose and insulin levels.

So is fat really the sixth taste? Mounting evidence indicates a strong possibility. However, several questions remain to be answered before a definitive conclusion can be made. For example, what are the precise roles of CD36, GPR120, and other receptors proposed to detect or modulate the fat taste? How, exactly, does fatty acid chain length influence taste perception? And are there specialized types of taste buds that detect the fat taste, as there are for other basic tastes?

Interestingly, lean mice and humans appear to have more fatty acid receptors on their taste buds and are therefore more sensitive to fatty acids than obese individuals, which reduces their propensity to overeat. “In other words, the more you taste fat, the less fat you eat,” write Keast and Costanzo (<http://dx.doi.org/10.1186/2044-7248-4-5>, 2015). In addition, diet appears to modulate the perception of fat: A high-fat diet decreases sensitivity, whereas a low-fat diet has the opposite effect. “The next 5 to 10 years should reveal, conclusively, whether fat can be classified as the sixth taste, but no matter what, there appears to be a functional significance to oral chemosensing of fats,” say the authors.

Information

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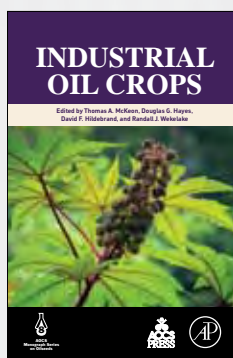
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Olio is produced by Inform’s associate editor, Laura Cassidy. She can be contacted at laura.cassiday@aocs.org.



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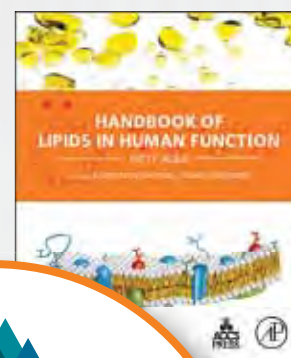
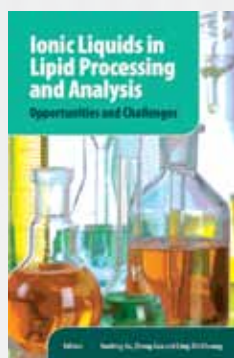
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Journal of the American Oil Chemists' Society (February)

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PATENTS

Delivery and controlled release of encapsulated lipophilic nutrients

Kohane, D.S. *et al.*, Pepsico Inc, US9186640, November 11, 2015

A complex coacervate delivery system is provided which encapsulates lipophilic nutrients such as, for example, fish oils high in omega-3 fatty acids. The complex coacervate delivery system protects the lipophilic nutrient from degradation, e.g., oxidation and hydrolysis, and also reduces or eliminates the unpleasant taste and odor of the lipophilic nutrient. The complex coacervate delivery system upon ingestion is operative to substantially release the lipophilic nutrient in the lower gastrointestinal tract in a pH-controlled manner. The complex coacervate delivery system may be included in a food or beverage product having a pH value within the range of about 1.5 to about 5.0.

Substance for dyeing keratin fibers, including cationic anthraquinone dyes and fatty acid triglycerides

Weser, G. and C. Kolonko; Henkel and Co Kgaa Ag, US9192556, November 24, 2015

The present disclosure provides an agent for coloring keratinic fibers comprising, in a cosmetic carrier (a) at least one compound of formula (I) and (b) at least one fatty acid triglyceride. The present disclosure also provides a method of using such an agent to produce increased shine, an intense color result with improved fastness properties, or reduced selectivity.

Thermochromic material, molded article comprising said material and use thereof

Loetzsch D., *et al.*, Fraunhofer Ges zur Foerderung der angewandten Forschung eV, US9193863, November 11, 2015

The invention relates to a thermochromic material comprising at least one biopolymer, at least one natural dye and at least one reaction medium, selected from the group of fatty acids and derivatives thereof, gallic acid and derivatives thereof and mixtures thereof. The thermochromic material according to the invention is completely based on non-toxic, natural products. Processing into materials or molded articles can occur, according to the invention, by means of conventional extrusion technology in the form of flat film, blown film or sheets or multi-wall sheets. The thermochromic material can be used in particular in the food industry and medical technology.

Ink composition

Vasudevan S., *et al.*, Hewlett Packard Development Co., US9193884, November 24, 2015

An ink composition includes a colorant, a co-solvent, and an acid chosen from oleic acid, linoleic acid, undecanoic acid, dodecanoic acid, tridecanoic acid, and combinations thereof. The ink composition further includes lithium and water. An anti-evaporative layer forms, during uncapped non-use, at an interface between air and the ink composition in an orifice of a nozzle, thereby reducing evaporation of the water from the ink composition.

Method to develop high oleic acid soybeans using conventional soybean breeding techniques

Bilyeu, K.D., *et al.*, Curators of the University of Missouri; Secretary of Agriculture as US Gov., US9198365, December 1, 2015

The present invention is directed to a soybean plant with mutations in FAD2-1A and FAD2-1B. Moreover, the present invention is directed to seeds from said plants with altered ratios of monosaturated and polyunsaturated fats. In particular, the present invention is directed to plants where the plants exhibit elevated levels of oleic acid.

Composition containing a cellulose, a vegetable oil and a volatile solvent, and use thereof as a dressing

Derain N., Laboratoires Urgo, US9198998, December 1, 2015

The present invention relates to a composition in the fluid form intended to form a dressing on the skin. This composition comprises from 6 percent to 12 percent by weight of the total weight of the composition of a cellulose derivative, from 5 percent to 15 percent by weight of the total weight of the composition of a vegetable oil, and a volatile solvent. The oil/cellulose ratio by weight is between 0.8 and 1.5. This novel dressing base makes it possible to obtain a film on the skin having satisfactory resistances to water and to rubbing actions. The film is flexible and sufficiently comfortable, in particular when the dressing is applied to a fairly extensive area of the skin.

Omega-7-rich compositions and methods of isolating omega-7 fatty acids

Shinde S., *et al.*, Heliae Dev LLC, US9200236, December 1, 2015

Disclosed here are compositions rich in omega-7 fatty acids, including palmitoleic acid, and products rich in omega-7 fatty acids derived from algal biomass. The algae and/or compositions rich in omega-7 fatty acids may be used in products or as ingredients of products. Methods and systems for increasing the production or concentration of omega-7 fatty acids, and isolating omega-7 fatty acids from algal biomass are also disclosed herein.

Feed additive composition for ruminants and method of producing the same

Hidetsugu, N.; Ajinomoto Co., Inc., US9204660, December 8, 2015

A feed additive composition includes a protective agent, lecithin in an amount of 0.05 to 6 percent by weight relative to a total weight of the composition, a basic amino acid in an amount of at least 40 percent by weight and less than 65 percent by weight relative to the total weight of the composition, and water. A method of producing a feed additive composition includes preparing a molten mixture of at least one protective agent, lecithin and at least one basic amino acid, and solidifying the molten mixture by immersing the molten mixture in water or an aqueous liquid. The protective agent includes hydrogenated vegetable oils and/or hydrogenated animal oils having melting points of greater than 50 degrees centigrade and less than 90 degrees centigrade.

Use of renewable oil in hydrotreatment process

Ouni, T., *et al.*, Neste Oil Oyj, US9206092, December 8, 2015

The use of bio oil from at least one renewable source in a hydrotreatment process, in which process hydrocarbons are formed from said glyceride oil in a catalytic reaction, and the iron content of said bio oil is less than 1 w-ppm calculated as elemental iron. A bio oil intermediate including bio oil from at least one renewable source and the iron content of said bio oil is less than 1 w-ppm calculated as elemental iron.

Method for cold stable biojet fuel

Seames, W.S., and T. Aulich, University of North Dakota, US9206367, December 8, 2015

Plant or animal oils are processed to produce a fuel that operates at very cold temperatures and is suitable as an aviation turbine fuel, a diesel fuel, a fuel blendstock, or any fuel having a low cloud point, pour point or freeze point. The process is based on the cracking of plant or animal oils or their associated esters, known as biodiesel, to generate lighter chemical compounds that have substantially lower cloud, pour, and/or freeze points than the original oil or biodiesel. Cracked oil is processed using separation steps together with analysis to collect fractions with desired low temperature properties by removing undesirable compounds that do not possess the desired temperature properties.

Process for the production of a fatty alcohol from a fatty acid

Cassidy, P.J., *et al.*, Johnson Matthey Davy Technologies Ltd, US9212114, December 15, 2015

In a process for producing fatty alcohol, fatty acid is subjected to esterification with a lower alkanol to form a stream of lower alkyl fatty acid ester(s). The stream is vaporized and then subjected to hydrogenation. The stream is then subjected to transesterification in a wax ester reactor to convert at least a portion of the lower alkyl fatty acid ester(s) to lower alkanol and wax ester(s). The resulting stream is then separated to yield a fatty alcohol(s) stream, a wax ester(s) stream, and an overhead stream comprising fatty alkanol(s) and alkane. The overhead stream is reacted in a wax ester reactor to convert at least a portion of the lower alkyl fatty acid ester to lower alkanol and wax ester(s). The wax ester(s) formed from the alkane is separated, along with any water and/or lower alkanol present.

Cosmetic composition obtained by esterification of amyl alcohol or an isomer thereof and a natural vegetable oil fatty acid

Chi, J.H., and Bae, D.H., US9212123, December 15, 2015

Disclosed is a cosmetic composition containing, as an active ingredient, an ester oil obtained by the bonding and esterification of amyl alcohol or an isomer thereof and a fatty acid derived from a natural vegetable oil such as olive oil or coconut oil. The ester oil increases the viscosity and imparts stability to cosmetics while also having a far better moisturizing ability and a lighter feeling in use than silicone oil, and the invention therefore allows a naturally derived ester oil, which is relatively friendly to the human body, to be used as a fundamental base component of a cosmetic composition, in complete or partial substitution for silicone oil which has been used on account of the shortcomings of existing synthetic ester oils.

Method of improving mechanical strength of flexible polyurethane foams made from bio-based polyols, the polyol compositions utilized therein and the foams produced thereby

Kaplan, W.A., *et al.*, Stepan Company, US9212250, December 15, 2015

A method of improving the mechanical strength of polyurethane foams made from bio-based polyols, the polyol-containing compositions utilized in the method of the invention, and the polyurethane foams produced by the method of the invention are provided. The method of the invention involves the incorporation of aromatic polyester polyol in the polyol-containing composition used to manufacture the foams. In one embodiment, the aromatic polyester polyol is utilized in a polyol-containing composition which is employed in the manufacture of flexible polyurethane foam. In another embodiment, the aromatic polyester polyol transesterified with a natural oil-containing composition to form a transesterification reaction product that is utilized in a polyol-containing composition which is employed in the manufacture of flexible polyurethane foam. Flexible polyurethane foams of the invention exhibit substantial improvements in mechanical strength properties, such as tear strength, tensile strength and elongation relative to foams made from bio-based polyols such as soybean oil-based polyols.

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.

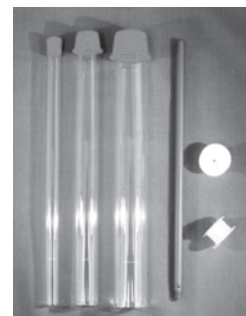


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Tips from inform|connect is a regular Inform column that features tips and other discussion highlights from the community forum board at <http://www.informconnect.org/home>.

Q: How do you measure the molecular mass of oil?

A: Animal- and plant-based oils and fats are composed of a mixture of triacylglycerol (TAG) species, each with its own molecular mass. For this reason, oils and fats do not have a single molecular mass. Rather, they have an average molecular mass that depends on the mass fraction of each TAG species in the oil. The molecular mass distribution and resulting average molecular mass can be determined using gel permeation chromatography and/or laser light scattering.

One community member pointed out that the molecular weight can be calculated from the composition (fatty acid content), but that a correction must be done in the case of free fatty acids or oligomers. Another member suggested a method (Picariello, 2007) that uses matrix-assisted laser desorption/ionization.

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Synthesis of fatty acid conjugates with phenols, carbohydrates, amines, and CH-acidic compounds by Pd(0)-catalyzed allylic substitution

Zobel, M. and H.J. Schäfer, *Eur. J. Lipid Sci. Technol.* 118 : 80–92, 2016, <http://dx.doi.org/10.1002/ejlt.201500195>.

The efficiency of a pharmaceutical can be increased by connecting it to a fatty acid. Nucleophilic allylic substitution of methyl 10-undecenoate and methyl oleate with palladium (0) as catalyst is explored to see, whether this reaction is useful for such connections with regard to yield and selectivity. For that purpose the allylic CH-bonds in methyl 10-undecenoate and methyl oleate were oxidized with selenium dioxide and *tert*-butylhydroperoxide. The obtained allylic alcohols were then activated by conversion into their methyl carbonates. Allylic substitution was performed with O-nucleophiles as methanol, carbohydrates, and different phenols as α -tocopherol and estradiol; furthermore N- and C-nucleophiles were applied. The yields of substitution products were in the range of 37–98%. High yields of conjugates were obtained with phenols, even when the hydroxy group was sterically shielded. These conjugates have an ether bond, a C–N or a C–C bond as link. They should have the advantage to be more stable in body fluids than the frequently applied conjugates being connected by the less inert ester bond. Triesters and dicyanoesters obtained with C-nucleophiles can be possibly used as cross linkers for polyesters and polyamides.

Distribution of animal drugs between skim milk and milk fat fractions in spiked whole milk: understanding the potential impact on commercial milk products

Hakk, H., *et al.*, *J. Agric. Food Chem.* 64: 326–335, 2016, <http://dx.doi.org/10.1021/acs.jafc.5b04726>.

Seven animal drugs [penicillin G (PENG), sulfadimethoxine (SDMX), oxytetracycline (OTET), erythromycin (ERY), ketoprofen (KETO), thiabendazole (THIA), and ivermectin (IVR)] were used to evaluate the drug distribution between milk fat and skim milk fractions of cow milk. More than 90% of the radioactivity was distributed into the skim milk fraction for ERY, KETO, OTET, PENG, and SDMX, approximately 80% for THIA, and 13% for IVR. The distribution of drug between milk fat and skim milk fractions was significantly correlated to the drug's lipophilicity (partition coefficient, $\log P$, or distribution coefficient, $\log D$, which includes ionization). Data were fit with linear mixed effects models; the best fit was obtained within this data set with $\log D$ versus observed drug distribution ratios. These candidate empirical models serve for assisting to predict the distribution and concentration of these drugs in a variety of milk and milk products.

Global adoption of genetically modified (GM) crops: challenges for the public sector

Huesing, J.E., *et al.*, *J. Agric. Food Chem.* 64:394–402, 2016, <http://dx.doi.org/10.1021/acs.jafc.5b05116>.

Advances in biotechnology continue to drive the development of a wide range of insect-protected, herbicide-tolerant, stress-tolerant, and nutritionally enhanced genetically modified (GM) crops, yet societal and public policy considerations may slow their commercialization. Such restrictions may disproportionately affect developing countries, as well as smaller entrepreneurial and public sector initiatives. The 2014 IUPAC International Congress of Pesticide Chemistry (San Francisco, CA, USA; August 2014) included a symposium on “Challenges Associated with Global Adoption of Agricultural Biotechnology” to review current obstacles in promoting GM crops. Challenges identified by symposium presenters included (i) poor public understanding of GM technology and the need for enhanced communication strategies, (ii) nonharmonized and prescriptive regulatory requirements, and (iii) limited experience with regulations and product development within some public sector programs. The need for holistic resistance management programs to enable the most effective use of insect-protected crops was also a point of emphasis. This paper provides details on the symposium discussion and provides background information that can be used in support of further adoption of beneficial GM crops. Overall, it emphasizes that global adoption of modern agricultural biotechnology has not only provided benefits to growers and consumers but has great potential to provide solutions to an increasing global population and diminishing agricultural land. This potential will be realized by continued scientific innovation, harmonized regulatory systems, and broader communication of the benefits of the high-yielding, disease-resistant, and nutritionally enhanced crops attainable through modern biotechnology.

Characterization of the key odorants in commercial cold-pressed oils from unpeeled and peeled rapeseeds by the sensomics approach

Pollner, G. and P. Schieberle, *J. Agric. Food Chem.* 64: 627–636, 2016, <http://dx.doi.org/10.1021/acs.jafc.5b05321>.

By application of aroma extract dilution analysis (AEDA) on the volatile fraction isolated from commercial cold-pressed rapeseed oil prepared from unpeeled seeds, 35 odor-active constituents in the flavor dilution (FD) factor range of 8–8192 were detected. The identification experiments showed that the earthy, pea-like-smelling 2-isopropyl-3-methoxypyrazine showed the highest FD factor of 8192, followed by 1-octene-3-one (FD 4096) and (*E,Z*)-2,6-nonadienal with an FD of 2048. After quantitation of the 16 key odorants showing FD factors ≥ 32 by stable isotope dilution assays and a determination of their odor thresholds in deodorized sunflower oil, odor activity values (OAV; ratio of concentration to odor threshold) could be calculated. The results indicated 2-isopropyl-3-methoxypyrazine, (*E,E*)-2,4-nonadienal (deep-fried, fatty), and (*E,Z*)-2,6-nonadienal (cucumber-like) with the highest OAVs. To confirm that the key aroma compounds were correctly identified and quantitated, a recombination experiment was performed by mixing the reference odorants in the same concentrations as

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CONTINUED FROM PAGE 42

they occurred in the rapeseed oil using odorless sunflower oil as the matrix. The recombinant showed a very good agreement with the overall aroma of the oil. In a commercial rapeseed oil prepared from peeled seeds, the same odorants were identified; however, in particular, the FD factor of dimethyl sulfide (DMS) was clearly higher. Quantitation of DMS in 10 commercial rapeseed oils from either peeled and unpeeled seeds revealed significant differences in DMS, but no influence of the peeling process on the amounts of DMS was found. The data can serve as a basis for the quality assessment of cold-pressed rapeseed oil.

Formation of plant sterol oxidation products in foods during baking and cooking using margarine without and with added plant sterol esters

Lin, Y., *et al.*, *J. Agric. Food Chem.* 64: 653–662, 2016, <http://dx.doi.org/10.1021/acs.jafc.5b04952>.

Plant sterols (PS) in foods are subject to thermal oxidation to form PS oxidation products (POP). This study measured POP contents of 19 foods prepared by typical household baking and cooking methods using margarines without (control) and with 7.5% added PS (as 12.5% PS-esters, PS-margarine). Median POP contents per portion size of cooked foods were 0.57 mg (range 0.05–1.11 mg) with control margarine versus 1.42 mg (range 0.08–20.5 mg) with PS-margarine. The oxidation rate of PS (ORP) was 0.50% (median) with the PS-margarine and 3.66% with the control margarine. Using the PS-margarine, microwave-cooked codfish had the lowest POP content, with 0.08 mg per portion, while shallow-fried potatoes had the highest POP content, 20.5 mg per portion. Median POP contents in cookies, muffins, banana bread, and sponge cake baked with the control or PS-margarine were 0.12 mg (range 0.11–0.21 mg) and 0.24 mg (range 0.19–0.60 mg) per portion, with a corresponding ORP of 1.38% and 0.06%, respectively. POP contents in all the cooked and baked foods did not exceed 20.5 mg per typical portion size. A wide variation in the distribution of individual POP among different foods existed, with 7-keto-PS and 5,6-epoxy-PS being the major oxidation products

Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies

Zhang, Y., *et al.*, *Am. J. Clin. Nutr.* 103: 330–340, 2016, <http://dx.doi.org/10.3945/ajcn.115.124081>.

The intake of fish and polyunsaturated fatty acids (PUFAs) may benefit cognitive function. However, optimal intake recommendations for protection are unknown. We systematically investigated associations between fish and PUFA intake and mild-to-severe cognitive impairment risk. Studies that reported risk estimates for mild cognitive impairment (MCI), cognitive decline, dementia, Alzheimer disease (AD), or Parkinson disease (PD) from fish, total PUFAs, total n-3 (ω -3) PUFAs, or at least one n-3 PUFA were included. Study characteristics and outcomes were extracted. The pooled RR was estimated with the use of a random-effects model meta-analysis. A dose-response analysis was conducted with the use of the 2-stage generalized least-squares trend program. We included

21 studies (181,580 participants) with 4438 cases identified during follow-up periods (2.1–21 y). A 1-serving/wk increment of dietary fish was associated with lower risks of dementia (RR: 0.95; 95% CI: 0.90, 0.99; $P = 0.042$, $I^2 = 63.4\%$) and AD (RR: 0.93; 95% CI: 0.90, 0.95; $P = 0.003$, $I^2 = 74.8\%$). Pooled RRs of MCI and PD were 0.71 (95% CI: 0.59, 0.82; $P = 0.733$, $I^2 = 0\%$) and 0.90 (95% CI: 0.80, 0.99; $P = 0.221$, $I^2 = 33.7\%$), respectively, for an 8-g/d increment of PUFA intake. As an important source of marine n-3 PUFAs, a 0.1-g/d increment of dietary docosahexaenoic acid (DHA) intake was associated with lower risks of dementia (RR: 0.86; 95% CI: 0.76, 0.96; $P < 0.001$, $I^2 = 92.7\%$) and AD (RR: 0.63; 95% CI: 0.51, 0.76; $P < 0.001$, $I^2 = 94.5\%$). Significant curvilinear relations between fish consumption and risk of AD and between total PUFAs and risk of MCI (both P -nonlinearity < 0.001) were observed. Fishery products are recommended as dietary sources and are associated with lower risk of cognitive impairment. Marine-derived DHA was associated with lower risk of dementia and AD but without a linear dose-response relation.

Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial

Chiu, S., *et al.*, *Am. J. Clin. Nutr.* online first, December 2015, <http://dx.doi.org/10.3945/ajcn.115.123281>.

The DASH (Dietary Approaches to Stop Hypertension) dietary pattern, which is high in fruit, vegetables, and low-fat dairy foods, significantly lowers blood pressure as well as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. The study was designed to test the effects of substituting full-fat for low-fat dairy foods in the DASH diet, with a corresponding increase in fat and a reduction in sugar intake, on blood pressure and plasma lipids and lipoproteins. This was a 3-period randomized crossover trial in free-living healthy individuals who consumed in random order a control diet, a standard DASH diet, and a higher-fat, lower-carbohydrate modification of the DASH diet (HF-DASH diet) for 3 wk each, separated by 2-wk washout periods. Laboratory measurements, which included lipoprotein particle concentrations determined by ion mobility, were made at the end of each experimental diet. Thirty-six participants completed all 3 dietary periods. Blood pressure was reduced similarly with the DASH and HF-DASH diets compared with the control diet. The HF-DASH diet significantly reduced triglycerides and large and medium very-low-density lipoprotein (VLDL) particle concentrations and increased LDL peak particle diameter compared with the DASH diet. The DASH diet, but not the HF-DASH diet, significantly reduced LDL cholesterol, HDL cholesterol, apolipoprotein A-I, intermediate-density lipoprotein and large LDL particles, and LDL peak diameter compared with the control diet. The HF-DASH diet lowered blood pressure to the same extent as the DASH diet but also reduced plasma triglyceride and VLDL concentrations without significantly increasing LDL cholesterol. This trial was registered at clinicaltrials.gov as NCT01404897.

Tomato peel powder as fat replacement in low-fat sausages: formulations with mechanically crushed powder exhibit higher stability than those with airflow ultra-micro crushed powder

Wang, Q., et al., *Eur. J. Lipid Sci. Technol.* 118: 175–184, 2016, <http://dx.doi.org/10.1002/ejlt.201400579>.

This study investigated the effect of addition of tomato peel powder, with different particle sizes as fat replacement, on the fatty acid composition and textural properties in low-fat sausage for 48 days. Results indicated that the hardness of low-fat sausages with no tomato powder (LFN) was significantly higher ($P < 0.05$) than that of the other groups, and springiness decreased slightly at a higher powder level. During storage, the polyunsaturated/saturated fatty acid ratio of high-fat samples with no powder (HFN) and low-fat samples with no powder (LFN) decreased significantly relative to that of the other sausage samples ($P < 0.05$). Furthermore, a slight increase in yellowness was detected in most samples. Low-fat sausage with powder prepared by airflow ultra-micro crushing had lightness and redness values of 54.26 and 11.36, respectively. Thus, these values can be increased effectively by adding a small amount of tomato peel powder treated by the airflow ultra-microcrushing method.

Aqueous enzymatic extraction and demulsification of camellia seed oil (*Camellia oleifera* Abel.) and the oil's physicochemical properties

Fang, X., X. Fei, H. Sun, and Y. Jin, *Eur. J. Lipid Sci. Technol.* 118: 244–251, 2016, <http://dx.doi.org/10.1002/ejlt.201400582>.

An aqueous enzymatic extraction method was developed to extract oil from camellia seed (*Camellia oleifera* Abel.). Individual enzymes and combinations of enzymes, pH, the ratio of material to water, reaction time and methods of demulsification were studied. The effects of hexane and aqueous enzymatic extraction on the oil's physicochemical properties (acid value, peroxide value, fatty acid profile, phenolics, and phospholipids content) were compared. The combination of protease/cellulase yielded significantly more oil than did other combinations. Under the optimal reaction conditions, a free oil yield of 82.37% was obtained. Four types of demulsification methods were compared. After demulsification with 20% ethanol (v/v), the highest total free oil yield of 91.38% was achieved. Compared with hexane-extracted oil, aqueous enzymatic-extracted oil was more acidic; had a higher percentage of monounsaturated fatty acid and contents of vitamin E and squalene; and had lower peroxide values, percentage of total saturated fatty acid, polyunsaturated acid, and content of both total phenolics and total phospholipids.

Electrochemical oxidation of cholesterol: An easy way to generate numerous oxysterols in short reaction times

Weber, D., Z. Ni, D. Vetter, R. Hoffmann, and M. Fedorova, *Eur. J. Lipid Sci. Technol.* 118: 325–331, 2016, <http://dx.doi.org/10.1002/ejlt.201500101>.

Oxysterol species are formed *in vivo* by enzymatic and non-enzymatic oxidation of cholesterol. Oxysterols were shown not only to be intermediates in the biosynthesis of bile acids and steroid hormones but also to possess versatile bioactivities. Many functions of oxysterols are not fully understood, others may not have been discovered yet, especially those of non-enzymatic origin. The limited accessibility to standard compounds challenges both analytics and functional studies. Here an amperometric flow-through electrochemical (EC) oxidation of cholesterol was applied to generate numerous oxidation products within short reaction times. Besides nine oxysterols confirmed with standard compounds (LC-MS), more than 10 additional, not identified oxidation products were present. The oxidation sites of the identified compounds were in agreement to the expected accessibility of the cholesterol backbone to free radical driven oxidation. Additionally, the presence of three products which are known to be synthesized enzymatically *in vivo* demonstrated a successful mimicking of these processes by EC as well. Several of the unidentified species showed the same analytical behavior (retention time and MS/MS) as compounds observed in extracts from a cardiomyocyte cell model of nitrosative stress. Further investigation of electrochemically generated compounds will allow identification and characterization of new oxysterols *in vivo*.

Analysis of long-chain unsaturated fatty acids by ionic liquid gas chromatography

Weatherly, C.A., et al., *J. Agric. Food Chem.* 64: 1422–1432, 2016, <http://dx.doi.org/10.1021/acs.jafc.5b05988>.

Four ionic liquid (IL) columns, SLB-IL59, SLB-IL60, SLB-IL65, and SLB-IL111, were evaluated for more rapid analysis or improved resolution of long-chain methyl and ethyl esters of omega-3, omega-6, and additional positional isomeric and stereoisomeric blends of fatty acids found in fish oil, flaxseed oil, and potentially more complicated compositions. The three structurally distinct IL columns provided shorter retention times and more symmetric peak shapes for the fatty acid methyl or ethyl esters than a conventional polyethylene glycol column (PEG), resolving *cis*- and *trans*-fatty acid isomers that coeluted on the PEG column. The potential for improved resolution of fatty acid esters is important for complex food and supplement applications, where different forms of fatty acid can be incorporated. Vacuum ultraviolet detection contributed to further resolution for intricate mixtures containing *cis*- and *trans*-isomers, as exemplified in a fatty acid blend of shorter chain C18:1 esters with longer chain polyunsaturated fatty acid (PUFA) esters.

CONTINUED ON PAGE 46

Potential of microbubbles as fat replacer: effect on rheological, tribological and sensorial properties of model food systems

Rovers, T., et al., *J. Texture Stud.*, in press, 2016, <http://dx.doi.org/10.1111/jtxs.12175>.

The potential of microbubbles as fat replacers and texture modifiers was assessed by comparison of the rheological and tribological properties of model food systems that contained 1) microbubbles 2) emulsion droplets, or 3) no added colloidal structures. We used a) liquids with thickener, b) liquids without thickener and c) gels as model food systems. A sensory test was performed in which we investigated whether panelists could discriminate between the different samples. It was found that the food system containing emulsion droplets had better lubrication properties than the dispersions containing microbubbles and solutions without any colloidal structures. The systems with emulsion droplets could be well discriminated sensorially from those with microbubbles or those without added colloidal structures. Samples containing a mixture of emulsion droplets and microbubbles were comparable to those of samples containing only emulsion droplets. We conclude that at the studied volume fraction of 5% the measured friction and perceived mouthfeel of systems containing microbubbles are rather different from those of systems containing emulsion droplets, while both have a diameter of about 1 μm . At this volume fraction, microbubbles cannot simply replace emulsion droplets.

Lipid Oxidation

Effect of magnesium salt concentration in water-in-oil emulsions on the physical properties and microstructure of tofu

Zhu, O., et al., *Food Chem.*, in press, 2016, <http://dx.doi.org/10.1016/j.foodchem.2016.01.065>.

The aim of this research was to prepare water-in-oil (W/O) emulsions encapsulating different concentrations of magnesium chloride (MgCl_2) and to investigate the effect of W/O emulsions on the physical properties and microstructure of tofu. The results showed that the stability of W/O emulsions improved as the concentrations of polyglycerol polyricinoleate (PGPR) and MgCl_2 increased. Dynamic viscoelastic measurements indicated that gelation time decreased with increasing MgCl_2 concentration in W/O emulsions, suggesting a more rapid reaction between magnesium ions and protein molecules. As the concentration of MgCl_2 in W/O emulsions increased, the yield and water content of tofu decreased, while the protein and crude fat contents and hardness values increased. At a concentration of 2.0 M MgCl_2 in W/O emulsion, the WHC and microstructure of the tofu samples were optimal. The variations in the physical properties of tofu were attributed to the concentration of magnesium ions and the coagulation rate.

Mechanical and water barrier properties of isolated soy protein composite edible films as affected by carvacrol and cinnamaldehyde micro and nanoemulsions

Otoni, C., et al., *Food Hydrocolloids*, in press, 2016, <http://dx.doi.org/10.1016/j.foodhyd.2016.01.012>.

Edible films may be used in food packaging, for which they must deliver good barrier and mechanical properties. Films based on proteins have good gas barrier and mechanical properties, but poor water barrier properties. Films made from lipids have good water barrier properties, but poor mechanical properties. Protein and lipids were then combined to form composite films, and the particle size of the lipid phase was reduced to evaluate its effect on the mechanical and barrier properties. Micro and nanodroplets of Acetem and Tween 60 were added into an isolated soy protein (ISP) solution. Oil-in-water droplets were formed by direct emulsification at 1,300 rpm for 30 min (microdroplets) or 5 h (nanodroplets), dispersed into the ISP solution, and cast into films. Emulsified films showed reduced strength and increased elongation, indicating a plasticizing effect of emulsions. The water barrier properties were either unchanged or slightly improved by the addition of hydrophobic compounds. Reducing droplet size improved the barrier properties as surface area of the lipid increased. Carvacrol and cinnamaldehyde were also added, and they either improved or unaffected the mechanical and barrier properties of ISP films. Addition of emulsions and reduction of droplet size generally decreased total pore volume in the films as well as water vapor permeation, indicating that the microvoid model suitably explains the water barrier properties of ISP films. This work further clarifies possible differences in barrier properties by examining water diffusivity and adsorption/desorption kinetics.

CONTINUED ON PAGE 48

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CONTINUED FROM PAGE 46

Combined chromatography and mass spectrometry for the molecular characterization of food emulsifiers

Du Chatinier, W. and H.-G. Janssen, *J. Chromatography A*, in press, 2016, <http://dx.doi.org/10.1016/j.chroma.2016.01.019>.

Food emulsifiers are widely used to stabilize water-fat emulsions such as mayonnaise and dressings. They are prepared by oligomerization of a poly-alcohol (as e.g. glycerol or citric acid) followed by a reaction with fatty acids. In order to gain insight in the chemical composition of different emulsifiers, a range of chromatographic methods including gas chromatography, size exclusion chromatography, normal phase- and reversed phase liquid chromatography either or not in combination with mass spectrometry was deployed. The different methods turned out to be highly complementary. By combining the information from different methods the polar head group and the fatty acid part of the emulsifier can be characterized in detail. Mass spectrometry is indispensable for establishing the number of polar molecules in the head group as well as for establishing the correct combinations of fatty acids in one molecule. Ten commercial emulsifiers were described at the level of number and type of polar groups and fatty acids present.

Novel technologies for the encapsulation of bioactive food compounds

Santiago, L. and G. Castro. *Current Opinion in Food Sci.*, in press. <http://dx.doi.org/10.1016/j.cofs.2016.01.006>.

Recent advances in nanoscience and nanotechnologies offer a revolutionary approach to the development of novel and healthier foods. Nano- and micro-technological carriers based on biomolecules may be tailored in order to deliver molecules, e.g., drugs, nutraceuticals and others, to any organ and tissue in the body. New micro- and nano-biocarriers based on food-grade polysaccharides, proteins, lipids and their blends, coacervates and also hybrid inorganic-biological molecules provide novel platforms to enhance the bioavailability, stability and delivery efficiency of bioactive molecules in the body.

Encapsulation of protein nanoparticles within alginate microparticles: impact of pH and ionic strength on functional performance

Zou, L., et al., *J. Food Eng.*, in press, <http://dx.doi.org/10.1016/j.jfoodeng.2016.01.010>.

Incorporation of bioactive proteins into functional foods is often challenging due to their instability to aggregation, sedimentation, or hydrolysis. In this study, core-shell protein nanoparticles, consisting of a zein core and a whey protein shell, were fabricated by antisolvent precipitation. The protein nanoparticles were then incorporated into biopolymer microgels fabricated by electrostatic complexation of casein and alginate. Protein nanoparticles were retained in microgels at low pH (3–5.5), but released at higher pH (6–7) due to microgel dissociation promoted by electrostatic repulsion between anionic casein and alginate. These microgels may be useful for retaining and protecting protein nanoparticles within acidic environments (e.g., stomach), but releasing them under neutral environments (e.g., small intestine). Protein nanoparticles were retained within microgels over a wide range of ionic strengths (0–2 M NaCl, pH 5). Protein nanoparticle encapsulation within microgels may improve their pH and salt stability in functional foods.

Potential applications of food derived bioactive peptides in management of health

Dhaval, A., et al., *Int. Journal Pept. Res. Ther.*, first online, February 2016, <http://doi.org/10.1007/s10989-016-9514-z>.

Recently the rise in noncommunicable diseases and side effects of drugs has promoted the research in food components with biologically active molecules. These bioactive components are vital in reducing and regulating the onset of such chronic degenerative diseases. Many food derived peptides are biologically active fragments encrypted within the primary protein sequence in nascent (inactive) form, hence also called 'cryptides'. These bioactive peptides range in size from 2 to 50 amino acids. They function beyond their basic nutritional benefits. Upon oral administration, these peptides play various roles such as opiate like, antioxidative, immunomodulatory, antihypertensive, hypocholesterolemic, mineral binding, antiobesity and antimicrobial. Both animal and plant proteins are rich sources of bioactive peptides having specific physiological and biochemical functions. Digestion of proteins in vivo or in vitro produces



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Apple peel flavonoids as natural antioxidants for vegetable juice applications

Massini, L., *et al.*, *Eur. Food Res. Technol.*, first online. February 2016, <http://doi.org/10.1007/s00217-016-2646-8>.

Commercial carrot and tomato juices (final concentration: 90 % juice, v/v) were added with a phenolic extract from apple peels consisting mostly of flavan-3-ols (>50 %), flavonol glycosides and dihydrochalcones in order to enhance their antioxidant capacity. The antioxidant contribution of the added extract to the capacity of the hydrophilic and lipophilic components of the juices was measured as ascorbic acid equivalents with ferric reducing-antioxidant power and radical scavenging capacity against DPPH[•] assays, and as inhibition against lipid peroxidation using an emulsified lipid in an oven test. Results showed that the addition of apple peel flavonoids at concentrations equal to or above 160 mg gallic acid equivalents (GAE)/L as total phenolics in the juices led to significantly higher ($p < 0.05$) radical scavenging capacity and to an increased protection against lipid peroxidation compared to control. The oxidative index of the model emulsified lipid with added enriched juices (20 mg/L as GAE) was lower than the control and comparable to a mixture of synthetic antioxidants (25 μ M). The antioxidant capacity of the enriched juices was mostly attributed to their hydrophilic components, particularly flavonoids with medium-to-high polarity such as catechins, dimers of (+)-catechin and (–)-epicatechin and quercetin glycosides. Nevertheless, it was suggested that oligomeric procyanidins with medium-to-low polarity could also contribute to the total antioxidant capacity as lipophilic components.

Industrial Applications

Ultrasound-induced green solvent extraction of oil from oleaginous seeds

Sicaire, A.-G., *et al.*, *Ultrason. Sonochem.* 31: 319–329, 2016, <http://dx.doi.org/10.1016/j.ultsonch.2016.01.011>.

Ultrasound-assisted extraction of rapeseed oil was investigated and compared with conventional extraction for energy efficiency, throughput time, extraction yield, cleanness, processing cost and product quality. A multivariate study enabled us to define optimal parameters (7.7 W/cm² for ultrasonic power intensity, 40 °C for processing temperature, and a solid/liquid ratio of 1/15) for ultrasound-assisted extraction of oil from oilseeds to maximize lipid yield while reducing solvent consumption and extraction time using response surface methodology (RSM) with a three-variable central composite design (CCD). A significant difference in oil quality was noted under the conditions of the initial ultrasound extraction, which was later avoided using ultrasound

CONTINUED ON PAGE 51



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CONTINUED FROM PAGE 49

in the absence of oxygen. Three concepts of multistage cross-current extraction were investigated and compared: conventional multistage maceration, ultrasound-assisted maceration and a combination, to assess the positive impact of using ultrasound on the seed oil extraction process. The study concludes that ultrasound-assisted extraction of oil is likely to reduce both economic and ecological impacts of the process in the fat and oil industry.

Energy requirements for wet solvent extraction of lipids from microalgal biomass

Martin, G.J.O., *Bioresour. Technol.* 205: 40–47, 2016, <http://dx.doi.org/10.1016/j.biortech.2016.01.017>.

Biofuel production from microalgae requires energy efficient processes for extracting and converting triacylglyceride lipids to fuel, compatible with coproduction of protein feeds and nutraceuticals. Wet solvent extraction involves mechanical cell rupture, lipid extraction via solvent contacting, physical phase separation, thermal solvent recovery, and transesterification. A detailed analysis of the effect of key process parameters on the parasitic energy demand of this process was performed. On a well-to-pump basis, between 16% and 320% of the resultant biodiesel energy was consumed depending solely on the process parameters. Highly positive energy balances can be achieved, but only if a correctly designed process is used. This requires processing concentrated biomass (ca 25% w/w) with a high triacylglyceride content (ca 30% w/w), and an efficient extraction process employing a non-polar solvent, low solvent-to-paste ratio, and efficient energy recovery. These requirements preclude many laboratory scale processes and polar co-solvents as viable options for large-scale biofuel production.

Microalgae from domestic wastewater facility's high rate algal pond: lipids extraction, characterization and biodiesel production

Drira, N., A. Piras, A. Rosa, S. Porcedda, and H. Dhaouad, *Bioresour. Technol.* 206: 239–244, 2016, <http://dx.doi.org/10.1016/j.biortech.2016.01.082>.

In this study, the harvesting of a biomass from a high rate algal pond (HRAP) of a real-scale domestic wastewater treatment facility and its potential as a biomaterial for the production of biodiesel were investigated. Increasing the medium pH to 12 induced high flocculation efficiency of up to 96% of the biomass through both sweep flocculation and charge neutralization. Lipids extracted by ultrasounds from this biomass contained around 70% of fatty acids, with palmitic and stearic acids being the most abundant. The extract obtained by supercritical CO₂ contained 86% of fatty acids. Both conventional solvents extracts contained only around 10% of unsaturated fats, whereas supercritical CO₂ extract contained more than 40% of unsaturated fatty acids. This same biomass was also subject to direct extractive-transesterification in a microwave reactor to produce fatty acid methyl esters, also known as, raw biodiesel.

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Pennycress: What's the catch?

Rose Hales

Field pennycress (*Thlaspi arvense*), also known as stinkweed or frenchweed, couldn't be called new by any stretch of the imagination and is, in fact, an enthusiastic weed which has been incensing farmers and land owners for centuries. Part of the *Brassicaceae* family, related to mustard and canola, the reason for its oversight is, ironically, the same thing that lies behind its possible success: Pennycress is not edible, and cannot be used for food production, but grows quickly and easily in reasonably hostile conditions.

- In the running as a new biofuels feedstock is field pennycress.
- The crop is ideal for growing and harvesting in rotation with soybean and corn and has a high oil yield, but it still hasn't taken off.
- This article explores the reasons why.

So why is pennycress being considered for biofuels? Pennycress, like most weeds, is quite an unfussy plant and is happy growing just about anywhere. It doesn't require much work, indeed, the weed-like traits of this plant mean that once the seeds have been sown, no maintenance is required at all until harvest. Its flat, heart-shaped pods contain tiny black seeds with 36% oil. This is double the oil contained in soybeans and very similar to rape/canola, and is considered to be a high yield. The seeds also contain 32% protein meal, which is a viable animal feed.

In the form of oil, pennycress has attributes which make it a strong contender in the biodiesel market. A US Department of Agriculture (USDA) study published in 2010 expounded the outcome of research into pennycress' properties. As part of its study, the USDA's Agricultural Research Service (ARS) produced a small amount of biodiesel from pennycress oil and evaluated it. Commenting on the research, it was said: "All diesel-based oils start to gel when it's cold enough. So the cloud point, which is the temperature at which crystals become visible in the fuel, is a crucial factor in both biodiesel and petrodiesel production. Another important property is the pour point, the temperature at which the fuel fails to pour as a result of excessive solidification. The average cloud and pour points for field pennycress biodiesel were 14°F (−10°C) and −0.4°F (−18°C), respectively. These temperatures were well below the cloud and pour

points of soybean oil-based biodiesel." These findings indicate that pennycress-based biodiesel could be used in cold climates.

In addition the researchers observed that: "...field pennycress methyl ester characteristics, such as acid value, oxidative stability, cetane number, cold flow properties, viscosity, sulphur content, and phosphorous content, are all satisfactory under ASTM D6751 (Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels)."

Significantly, pennycress is not a food crop. Therefore using it to produce biofuel does not compete with food use, quashing the food versus fuel debate that has clouded so many potential oilseeds in the past. The debate is fixed upon the risks of diverting farmland suitable for producing food to biofuel production.

Also, in March 2015, the US Environmental Protection Agency (EPA) completed an analysis of the greenhouse gas (GHG) emissions attributed to the production and transportation of pennycress oil for biofuel production. The analysis determined that pennycress "is not expected to have significant indirect land use changes (ILUC)." It is a winter annual that is sown in the early autumn and harvested in late spring, meaning it is not in competition with the rotations of soybean or corn, and can be grown while the fields would otherwise be empty, as exemplified in Fig. 1.



FIG. 1. Example of soybean, corn, and pennycress rotation. Compiled by the Environmental Protection Agency. Source: Excerpted from Fig. 2 in "A life cycle assessment of pennycress (*Thlaspi arvense* L.)-derived jet fuel and diesel," Biomass and Bioenergy (<http://dx.doi.org/10.1016/j.biombioe.2012.12.040>, 2013).

Even better, growing pennycress actually benefits the food crops it is grown in rotation with; it acts as a cover crop, preventing erosion and nitrogen leakage into ground water, as it uses the leftover nitrogen from the corn crop.

To produce oil from the seeds, only the traditional and highly available production, logistics, and processing equipment is required. Seeds need to be crushed using a screw press (or full press)—high pressure from an expeller squeezes the oil from the seeds leaving behind a press cake. This process is less complex than soybean oil extraction or ethanol production. USDA research found that the oil can be converted to fatty acid methyl esters (FAME) using a sodium methoxide catalyst in methanol.

WHAT'S THE CATCH?

Even as a consideration for biofuel, the plant is not new. Reports have been appearing for years, quietly evangelizing the positive attributes of so-called “stinkweed.” So what’s holding it back? The simplest reason why pennycress is still on the side lines of the biodiesel industry is cost. Growing the crop is not yet economically viable and thus persuading farmers to cultivate it is difficult. Pennycress is caught in a “catch 22”—it will only become cost effective once it is grown in large quantities but no one is willing to commit to growing it until it is clear that it can make money.

An answer to this problem is, however, already on its way from a young US biotech firm called Arvegenix. Arvegenix may only be three years old, but it’s got experience on its side. Jerry Steiner, the company’s founder, is a former Monsanto executive, and many other members of the modest team also bring decades of knowledge to the table. In 2013, Arvegenix had only 300 plots being tested under field conditions. The number of plots grew to over 2,000 in 2014, and now the team has over 6,000 plots planted at a range of different locations.

An article published in February 2015 in the *Seattle Times* reports on the company’s research. Arvegenix is attempting to domesticate a wild strain of pennycress (just as soybean was domesticated in the 1920s). The company is aware of the qualities of pennycress, but it also has a strong understanding of what needs to be done to make a future with pennycress-based biodiesel a reality; it is focusing on “using advanced breeding technology to nudge the plant toward something better than it is today. A plant needs to be more predictable, more consistent and one that produces a higher oil yield.” Achieve these simple goals and the crop will have “no trouble attracting the attention of farmers,” Dennis Plummer told the *Seattle Times*. Arvegenix is hoping to achieve domestication of the crop by the end of this decade.

According to a report in the *St. Louis Business Journal* in May 2015, Arvegenix has succeeded in raising US\$2.5 million from a group of investors including Monsanto, Cultivation Capital, and BioGenerator to be used to expand the R&D program, as well as fund regulatory studies and grow operations.

ONCE A WEED, ALWAYS A WEED

The final hurdle facing the large-scale production of pennycress is that it is currently listed on the restricted weed list in nine prospective growing-states of the northern United States, including Michigan and Iowa. The EPA’s report on the crop notes that this

indicates “limitations on the use of the plant in those [states] and a high probability of impacting production systems such as agriculture, nurseries and forest plantations.”

Many farmers may need convincing that pennycress isn’t going to become invasive. *Biodiesel Magazine* interviewed Terry Isbell, lead researcher in the new crops and processing technology group at the National Center for Agricultural Utilization Research in Peoria, Illinois, who explained: “Farmers will be concerned that the plant will take over their fields.” Corn and soybean’s ordinary herbicide programs should control the spread of pennycress. However, if rotated with other winter annuals, he admits a problem could be created during that season (not affecting summer crops).

GREENHOUSE GAS EMISSIONS

Pennycress is clearly becoming an oilseed to watch, and in just a few short years it could be at a stage where planting the winter annual is the more popular choice.

In preparation, Arvens Technology—pennycress developers seeking to produce biofuel and aviation jet fuel from the crop—filed a petition with the EPA, leading it to undertake the previously mentioned analysis of the crop’s GHG emissions. The report thoroughly examined the production and transportation of the oil and noted that: “new agricultural sector modelling is not needed to evaluate the lifecycle GHG impacts of using pennycress oil as a biofuel feedstock for purposes of making GHG reduction threshold determinations for the RFS program. This is in part because of the similarities of pennycress oil to soybean oil and camelina oil, and because pennycress is not expected to have significant land use change impacts.” Instead of performing new agricultural sector modelling, EPA relied upon the soybean oil analysis conducted for the March 2010 rule to assess the relative GHG impacts of growing and transporting pennycress oil for use as a biofuel feedstock.

The 2015 report additionally looked into the future of pennycress cultivation and what could be achieved. As the crop can be rotated with soybeans the USDA determines that “pennycress could be cultivated on 31 million acres in Illinois, Iowa, Ohio, and Indiana”—current soybean growing spaces. “However, industry is also considering cultivating pennycress in other Midwest corn-belt states and, according to their estimates, 40 million acres could be cultivated,” the report added.

The report is now closed for comments, which the EPA will review, combining them with its own evaluations of GHG emissions associated with the agricultural use of pennycress oil feedstock and emissions attributed to producers’ production processes to “determine whether the proposed pathways satisfy CAA lifecycle GHG emissions reduction requirements for RFS-qualifying renewable fuels.” The report concluded that it anticipates biofuel produced from pennycress “could qualify as biomass-based diesel or advanced biofuel (when using typical fuel production processes).”

Rose Hales is editorial assistant at Oils & Fats International.

This article was reprinted with permission from the July/August 2015 issue of *OFI* magazine and updated by *Inform* staff.

Uruguayan beef tallow and its applications

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

Leslie Kleiner

Uruguay, like Argentina, has a tradition of raising cattle on pastures. To understand the applications of Uruguayan beef tallow arising from pasture raised cattle, I interviewed Maria Antonia Grompone, director of the fats and oils laboratory, at the Chemistry School of Universidad de la República, Uruguay.



Q: How does Uruguayan beef tallow differ from that produced in other countries?

A: Uruguayan beef tallow is different in its fatty acid composition and physical properties from beef tallow produced in other countries. Although its palmitic acid content is similar to other beef tallow (20–27%), its stearic acid content tends to be higher (25–34%), and its oleic acid content lower (31–43%) than that found in beef tallow from other regions. Therefore, its melting point is generally higher (> 48 °C) than that of other beef tallows. This is probably due to differences in agricultural and husbandry practices among the regions. In Uruguay (like Argentina), cattle is usually free to exercise in the prairies and feed from the pastures.

Q: Are there differences in tallow arising from grass-fed versus grain-fed cattle?

A: Beef from free-range, pasture-fed cattle (like those produced in Uruguay) has a lower amount of intramuscular and extramuscular fat than cattle raised in confinement and with a grain- and hay-based diet. This difference does not seem to be heavily influenced by the animal breed. For example, the main breed for meat production in Uruguay is “Hereford,” while in Argentina it is the “*Aberdeen Angus*.” Although diet has a role on fat composition, the variation within this fat composition is small. There is a slight influence of grain-based feed (which is very rich on unsaturated fatty acids) on fat composition. The process of bio-hydrogenation, which occurs in the animal’s rumen, considerably lowers the unsaturated fatty acid content of the total fat composition.

Q: What are common uses of tallow in Uruguay and Mercosur?

A: The most common use of beef tallow in Uruguay and Argentina is found in bakery applications. Although beef tallow can also be used for fried applications, its applications in this area have been diminishing due to the use of partially hydrogenated oils and/ or high-oleic oils. However, beef tallow is still an essential component for traditional fried foods such as “*tortas fritas*.” These are a typical Uruguayan food comprised of a non-leavened dough that is then most commonly fried in beef tallow.

In non-food applications, beef tallow is used for biodiesel production. However, at temperatures that are not too low (~15 °C), beef tallow can start to gel or form crystals. For this reason, it may be convenient to mix beef tallow with vegetable oils before methylating for biodiesel production.

Q: How does the oxidative stability (as measured by OSI) compare to the most commonly used frying oils in Uruguay?

A: Due to its high saturated fatty acid content, beef tallow has a high oxidative stability. Beef tallow’s induction time for OSI measured at 110 °C can reach up to 38 hours, in comparison to 20 hours for refined, bleached and deodorized (RBD) high-oleic sunflower oil, which is commonly used for frying food in Uruguay. However, the quality of commercially available beef tallow in Uruguay is quite variable, and depending on the brand some barely reach 5 hours of induction time (this is comparable to RBD high-linoleic sunflower oil, which is not stable enough for frying).

Q: How does beef tallow influence mouthfeel in bakery applications?

A: The high saturated fatty acid content of beef tallow imparts a unique thermal behavior to bakery goods. At body temperature (temperature in the mouth) beef tallow contains ~30% solids, which is not ideal for all bakery applications. At this temperature and level of solids, some solids are not melted in the mouth and could therefore be perceived as having a waxy mouthfeel. To improve on this applications, beef tallow can be fractionated by controlled cooling (process currently in use in Argentina). Fractionating close to 40 °C, a liquid fraction (olein) can be obtained, and this fraction can then be used in bakery applications with an improved mouthfeel.

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



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Using INCI for cosmetic back-label assignment

Eric S. Abrutyn

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

The *International Cosmetic Ingredient Dictionary & Handbook* has been in existence since the early 1970s. It has since evolved into a global compendium for naming cosmetic ingredients for back label disclosure and referencing in technical journals. This voluntary self-regulated industry approach was accepted by the US Federal Drug Administration (FDA), and thus cited as the second source for names for ingredient declaration.

Even with this comprehensive recognition and use of INCI nomenclature worldwide, there are still misunderstandings as to how the assignment process works and assigned names really mean from a regulatory perspective. This article is aimed at members of the scientific community who are not directly familiar with cosmetic ingredient nomenclature and may find the name assignments confusing, inappropriate, inconsistent with International Union of Pure and Applied Chemistry (IUPAC) or Chemical Abstracts Service (CAS) protocols.

Let's begin by defining some terms that have become commonplace. INCI is defined as International Nomenclature Cosmetic Ingredient. The part of the compendium where the monographs for ingredients are compiled in alphabetical order by INCI name is the dictionary. Each monograph contains pertinent information about the ingredient: its chemical composition; CAS/European Inventory of Existing Commercial Chemical Substances (EINEC) numbers databases; citations in the US Code of Federal Regulations (CFR), EU regulations, Merck Index, Over-the-Counter Drugs (OTC), Cosmetic Ingredient Review (CIR), Toxic Substances Control Act (TSCA), etc.; Japanese and Chinese translations; chemical and trade synonyms; and product category, function, and source information. Much of this information is conveniently cross-referenced in the handbook portion, along with additional regulatory information.

The International Nomenclature Committee (INC) has focused on keeping up with the growing changes and complexity of chemistry, without compromising the scope and intent of the dictionary. The dictionary's primary objective is to provide consumers with an easy to understand, referenced identification of the ingredients they apply to their bodies.

This identification should also inform members of the medical profession, so they can properly advise patients about what ingredients or products they should use or avoid.

A COMPENDIUM OF 17,000 INGREDIENTS AND STILL GROWING

Much has transpired since the 1st Edition was published. The name of the book morphed into an international dictionary and handbook recognized all over the world as the official compendium of approved cosmetic ingredient nomenclature. The compendium has grown to represent over 17,000 ingredients and 60,000 trade-names, requiring four 2-inch-thick books just to hold all the information. The European Union, Japan, and China eventually recognized the Personal Care Products Council (PCPC) INCI nomenclature for the declaration of ingredients. Additionally, countries with a regulatory position for personal care products (Argentina, Canada, Colombia, Israel, Republic of Korea, Mexico, Norway, Saudi Arabia, South Africa, Bulgaria, Brazil, Costa Rica, Lithuania, Malaysia, The Philippines, Poland, Russian Federation, Singapore, Switzerland, and Thailand, and those just starting to establish regulations) also consider this dictionary/handbook as an official compendium.

The INC has a key responsibility to uphold the integrity and validity of the data incorporated into the compendium. Without continued maintenance, the compendium would lose validity worldwide. The current approach provides the best balance needed to rapidly provide the public with a comprehensive source of information about the ingredients they apply to their bodies. The INC continues to harmonize the data with other countries to broaden its acceptability and

applicability within a growing global consumer marketplace. The dictionary continues to be designed to provide consumers with a repository where straightforward and reliable information and references on cosmetic ingredients are readily available.

The FDA regulations on cosmetic labeling (21 CFR Part 701) include prescribed guidelines on how to define ingredients in a cosmetic product. Since the 2nd Edition was published in 1977, the INCI dictionary has been considered the primary source for names used in declaration of cosmetic ingredients for back labeling. In the absence of an assigned name in the INCI dictionary, the FDA has clearly established guidelines to allow names assigned from other acceptable sources (21CFR701.3(c)). Theoretically speaking, if there is no name assigned in the INCI dictionary, one could use a standard chemical nomenclature that is recognized by consumers. This is not common practice, since the process for getting an assigned name is relatively easy, and the FDA could frown on the use of a name that is not from one of the recognized sources outlined in the regulations.

There has been a general misunderstanding of what an INCI assigned nomenclature is and what it does and does not represent. The assignment of an INCI name and inclusion of an ingredient in the INCI dictionary is not an endorsement of safety or regulatory acceptance, or substantiated function for use as a cosmetic ingredient in the United States or any other country. The dictionary's only purpose is to provide a recognizably defined name for cosmetic ingredients, thus establishing

a common language for communicating the identity of ingredients on a global basis. Therefore, it is the responsibility of the user of those ingredients to do due diligence to insure safety and regulatory acceptability before incorporating any ingredient into a consumer personal care product. It is also the responsibility of the cosmetic company to make sure that the product has the correct assigned nomenclature and is representative of the definition of that ingredient. Consequently, it behooves cosmetic companies to require ingredient suppliers to supply letters assuring that the ingredient and tradename as supplied is in fact representative of the INCI assigned name. Further, the committee is not responsible for establishing the ingredient's functional use. Information about functional use is provided by the submitter and is not substantiated by the committee.

INCI names are assigned on the basis of the chemical composition of an ingredient, and the INC can only work from the information provided by the manufacturer; so, prescribed acceptable information needs to be clearly indicated on the INCI application. See Fig. 1 for a flow chart on the process used to assign a monograph from a Tradename (TN) application. Note that the committee assigns names that are representative of the chemical description of the raw material. Additionally, the committee will ensure that disclaimers are included in the definition of an ingredient purported to have a function that implies over-the-counter (OTC) drug status in the United States and specific regulatory status in other countries (such as the



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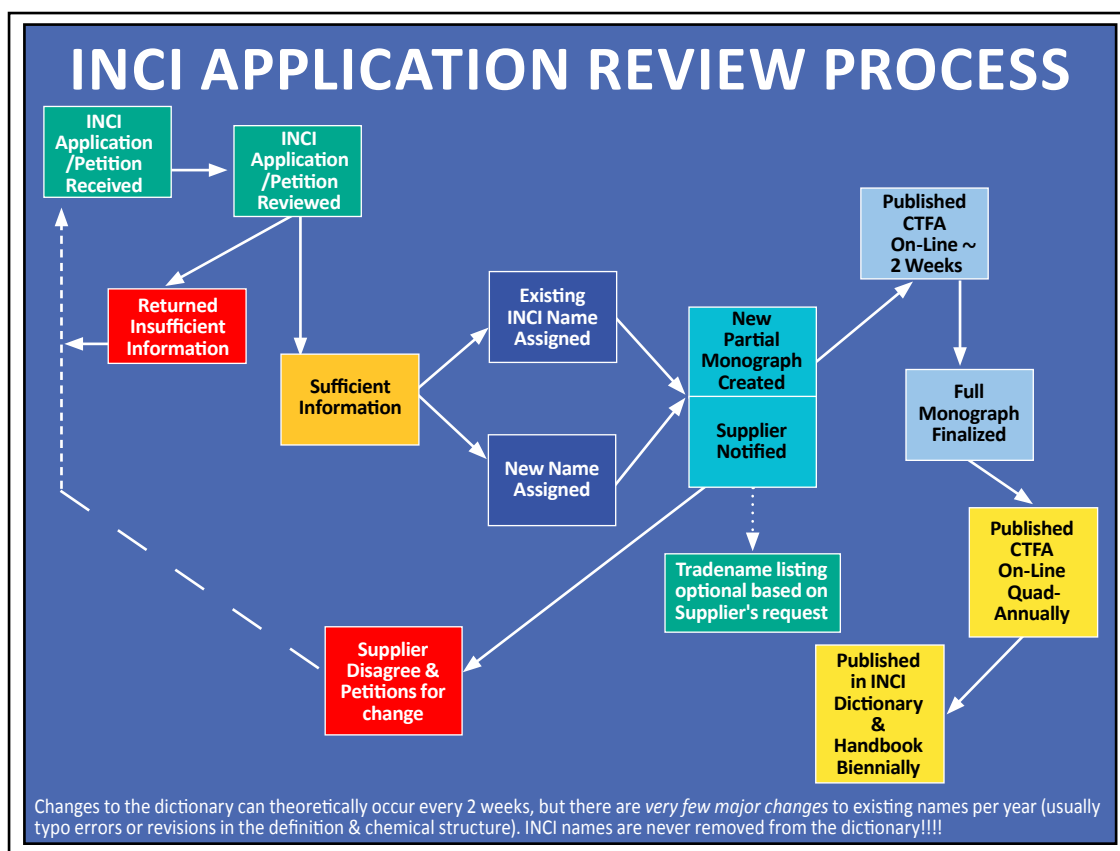
October 4–7, 2016. World Conference on Fabric and Home Care—Singapore 2016, Shangri-La Hotel, Singapore. <http://singapore.aocs.org>

April 30–May 3, 2017. AOCs Annual Meeting and Industry Showcases, Rosen Shingle Creek, Orlando, Florida, USA.

September 11–14, 2017. 17th AOCs Latin American Congress and Exhibition on Fats and Oils, Grand Fiesta Americana Coral Beach Hotel, Cancun, Mexico

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Fig. 1. INCI application process flowchart



citation of the function “skin protectant” for an ingredient that is not an OTC Category I Skin Protectant in the United States).

If during the process of making an ingredient, a filter aid, decolorizing/deodorizing activated carbon, or other component is introduced and subsequently removed, it is not considered part of the final composition. If ingredients are added and react to form another ingredient (for example, adding lactic acid and sodium hydroxide to make *in-situ* sodium lactate), the individual components do not have to be listed separately (except in polymer naming protocol); instead, the newly formed ingredient could be considered part of the composition. If the ingredient is present as a small residual component of the composition but does not contribute to its functionality, it usually does not become part of the final ingredient composition. Examples of situations in which non-functional residuals are not considered part of the blend/mixture composition include: (1) small amounts of preservative that allow for safe shipping and storage of the ingredient but represent a very small non-functioning part of the final composition; (2) small amounts of emulsifiers that keep the ingredient in suspension for ease of use and storage—although most companies today include suspension ingredients in the blend/mixture composition of the product nomenclature; and (3) unreacted starting ingredients or excess of one ingredient to drive the reaction to completion that are considered a minor portion of the composition—such ingredients are usually not considered as separate component of a blend/mixture, although this is a gray area.

The declaration of ingredients on cosmetic products back label ingredient statement lists ingredients in order of predominance. Ingredients that are equal to or below a concentration of 1% may be declared in any order. Colors are also listed in

random order at the end of the ingredient declaration. Whether the presence of a large amount of an incidental should be identified in the INCI name and/or declared in the ingredient listing is debatable. As a general rule, FDA advises that when there is doubt about whether the ingredient is truly incidental, the ingredient should be declared in the ingredient listing. Flavors and fragrances may be listed by these terms in their respective descending order. The labeling of OTC drugs and cosmetic drugs follow special requirements not covered in this article. In these cases, readers are directed to consult the appropriate OTC drug labeling regulations, or the CTFA Labeling Manual.

INC follows written conventions which are extensively covered in the Dictionary/Handbook Introduction in Volume 1 page xxii (as well as contained in the On-Line). These conventions are guidelines to assure consistency of assignments, although the committee occasionally modifies them or adds new ones. To help clarify how names are assigned, the key conventions are summarized on page 60.

Eric Abrutyn has more than 45 years of experience in the personal care cosmetics industry. He is a member of the Technical Advisory Board and past-contributing editor for Cosmetics & Toiletries magazine, a contributing author and instructor for SpecialChem4Cosmetics, and a member (and past chair) of the International Nomenclature Committee (INC)—part of Personal Care Products Council’s (formerly CTFA). Abrutyn founded the consulting firm TPC2Advisor’s Ltd., Inc, which provides technical support to clients in the cosmetics and personal care industries. He can be contacted at tpc2advisors@yahoo.com.

Summary of some key written conventions

1. All alkoxylated materials must be named by including the alkoxylation level as the average number of moles of ethylene oxide and/or propylene oxide (reference Conventions #17–19, page xxiii).

- a) If the alkoxylation is a mixture composition of EO and PO, then the order of addition is required and where they are reacted on the ingredient is necessary for proper nomenclature assignment.

2. Alkyl groups are typically named per the convention on page xxv (reference Conventions #20–25).

- a) If the alkyl comes from a natural source, the purity is important to determine if a chemical name or a source name is required (e.g., Cetearyl vs. Palm or Tallow).

3. Esters require good manufacturing and starting ingredient information to understand which convention applies.

- a) “Glycerides,” as a suffix term, refer to the reaction of fatty acids with glycerin to form a complex mixture of mono-, di-, and tri-ester linkages as outlined in Convention #38, page xxviii.
- b) Transesters are complex compositions derived by the process of transesterification of triglycerides, and are identified with the suffix “ester” as outlined in Convention #54, page xxxi (e.g., “Apricot Kernel PEG-8 Esters”).

4. Oils and Triglycerides

- a) “Oil” as a suffix descriptor is used for plant or animal origin triglycerides as outlined in Convention #42, page xxix.
- b) If these oils are further derivatized (e.g., hydrogenation, acetylation, oxidation), the process name goes first, but the “oil” suffix remains (e.g., “Hydrogenated Castor Oil”).

5. Polymers have become more complex over the past few years as new controlled processes are commercialized (reference Conventions #43–46, page xxix).

- a) They are named from the starting monomers in alphabetical order, with slashes (/) between each monomer.
- b) Homo-polymers are named with “poly” as the prefix
- c) “Copolymer” suffix is used when more than one monomer is used to make the polymer.
- d) When there are more than 4 monomers, the polymer class name is used with an arbitrary number (e.g., “Polyester-1”, “Polyquaternium-11”).
- e) “Crosspolymer” suffix is used when the polymer is crosslinked; only when the crosslinking ingredient is a polymer will it be included in the assigned nomenclature.

6. Botanicals require identification of the genus and species, along with detailed information on the process and final composition.

- a) Distillation will produce a water-soluble component (“water” suffix) and water-insoluble portion (“oil” suffix).
- b) Any extraction process (e.g., press, solvent extraction, etc) will be designated by an “extract” suffix, unless extraction was mechanical pressing, e.g., to obtain the juice from a fruit in which case a “juice” suffice applies.
- c) Harmonization has eliminated usage of common names in most cases.
- d) Water is simply designated as “water,” unless it is a fragrance water derived by steam distillation, or water derived from the sea which is termed “sea water.”

7. Minerals

- a) Minerals are typically named from either their source, geological name, or a well- documented common name.
- b) If the mineral is a synthetic replicate, then the assignment would be based on the inorganic materials used and its process, unless the final composition can be accurately proven as physically indistinguishable from the natural sourced composition.

8. Bio-active material have become quite complex and will not be covered in this article except for a few key points:

- a) Typical naming of materials from a biotech process requires identification of the bacteria used and complete details of the process.
- b) If the bacteria is pathogenic, then the genus and species are both necessary and will be part of the assigned nomenclature.
- c) If the gene, or any part of the composition, is human sourced (or a recombinant) then “Human” will be part of the assigned nomenclature.

9. Powder is a form and usually not denoted in the nomenclature assignment unless there is a clearly defined mechanical process resulting in a significantly reduced particle size.

Source: INCI Applications & Nomenclature conventions at <http://online.personalcarecouncil.org/jsp/Home.jsp>

Method of producing DHA-rich oil using lipase

Omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), are recognized as essential nutrition for human beings. Lipase produced by such microorganisms as *Aspergillus*, *Burkholderia*, *Candida*, *Pseudomonas*, and *Rhizomucor*, can concentrate these nutritional PUFAs in the natural form of “acylglycerols.” *Candida rugosa* lipase (CRL) is one of the most popular biocatalysts for this industrial application. This article describes the unique and key features of CRL.

Hirouchi Yuji and Doisaki Nobushige

- Lipase produced by *Candida rugosa* catalyzes hydrolysis to concentrate DHA in the acylglycerol fraction efficiently.
- Reaction at low temperatures yields DHA-rich oil that is lower in saturated fatty acids than that produced at higher temperatures.
- Removing cholesterol before lipase-catalyzed hydrolysis results in a DHA-rich oil with a lower content of cholesterol.

PRODUCING DHA-RICH OILS IN ACYLGLYCEROL FORM

PUFAs can be concentrated in the acylglycerol fraction in various ways. For example, “winterization” is a traditional process in which fatty acids with different saturations are separated based on their melting point. This is a very simple process in which low temperature enables the triacylglycerols (TAGs) comprising saturated fatty acids to precipitate. However, achieving the very low temperatures required to significantly increase PUFA contents consumes large amounts of energy. The PUFA yield also decreases due to poor separation of PUFAs from other unsaturated fatty acids.

In contrast, enzymatic method is energy efficient, since the reaction of lipase-catalyzed hydrolysis can occur at room temperature. PUFAs are concentrated efficiently by using fatty acid-specific lipase. On the other hand, the correct selection of lipase is crucial, since different types of lipase have different specificity to fatty acids. Because CRL acts extremely weakly on DHA, DHA is enriched in the glyceride fraction and the yield of DHA increases. It is important to note that this hydrolysis is an equilibrium reaction, and DHA concentration can only increase to a certain point. For example, when the hydrolysis of tuna oil that contains about 25% DHA is catalyzed by CRL, DHA is concentrated to about 45–50%, but it is difficult to achieve concentrations higher than 50%—even with a higher dose of CRL. More DHA-enriched oil can be obtained by re-hydrolyzing the DHA-rich oil fraction that separates from the reaction mixture (Fig. 1). DHA can be concentrated to more than 70% in the glyceride fraction by repeating the separation of DHA-rich oil fractions and hydrolysis processes. Most DHA-enriched oils used as health foods or dietary supplements in the Japanese market are processed with this way.

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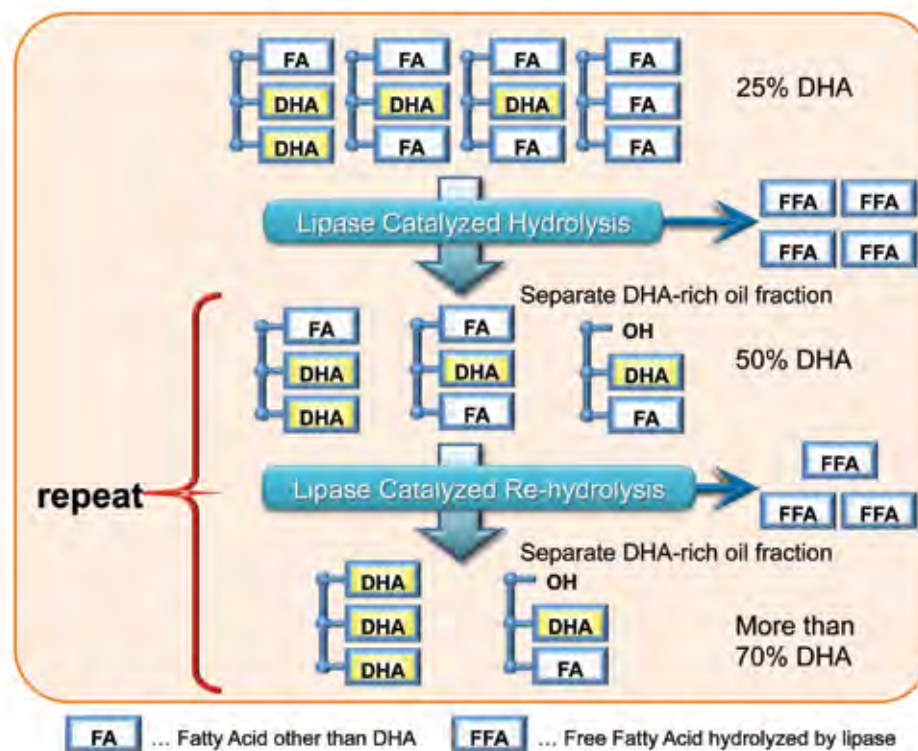


FIG. 1. DHA is enriched in the glyceride fraction efficiently, since *Candida rugosa* lipase acts extremely weakly on DHA. DHA concentration can only increase up to a certain point no matter how much lipase is dosed. Re-hydrolysis of DHA-rich oil fraction separated from the reaction mixture achieves higher concentrations of DHA.

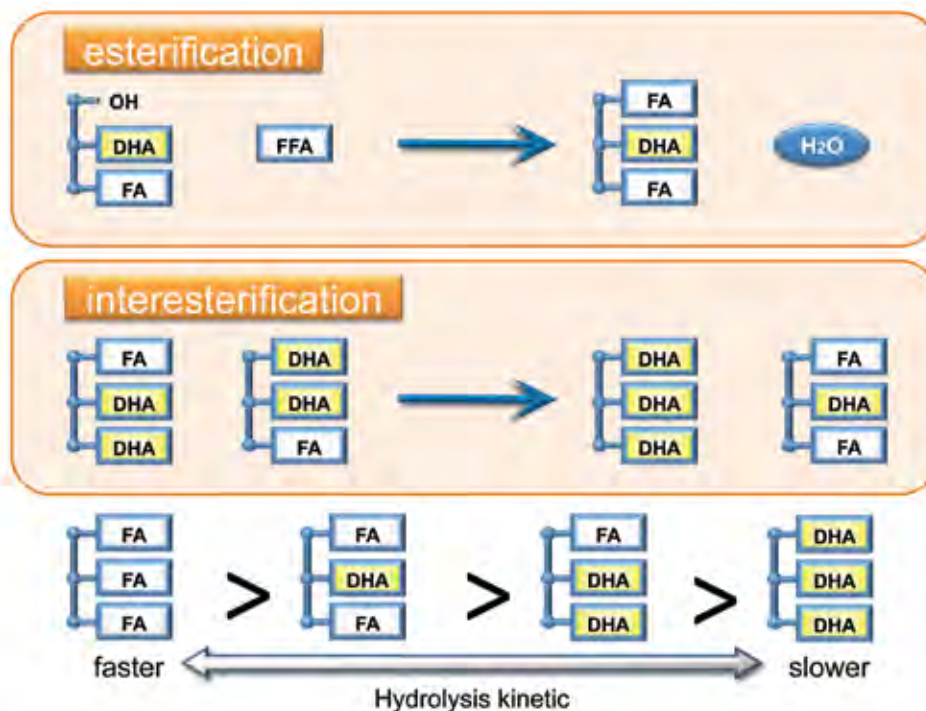


FIG. 2. The rearrangement of PUFAs esterified and interesterified by *Candida rugosa* lipase provides di-PUFA TAG and/or tri-PUFA TAG. Hydrolysis kinetics of TAG depend on the number of PUFA bound in glycerol moiety.

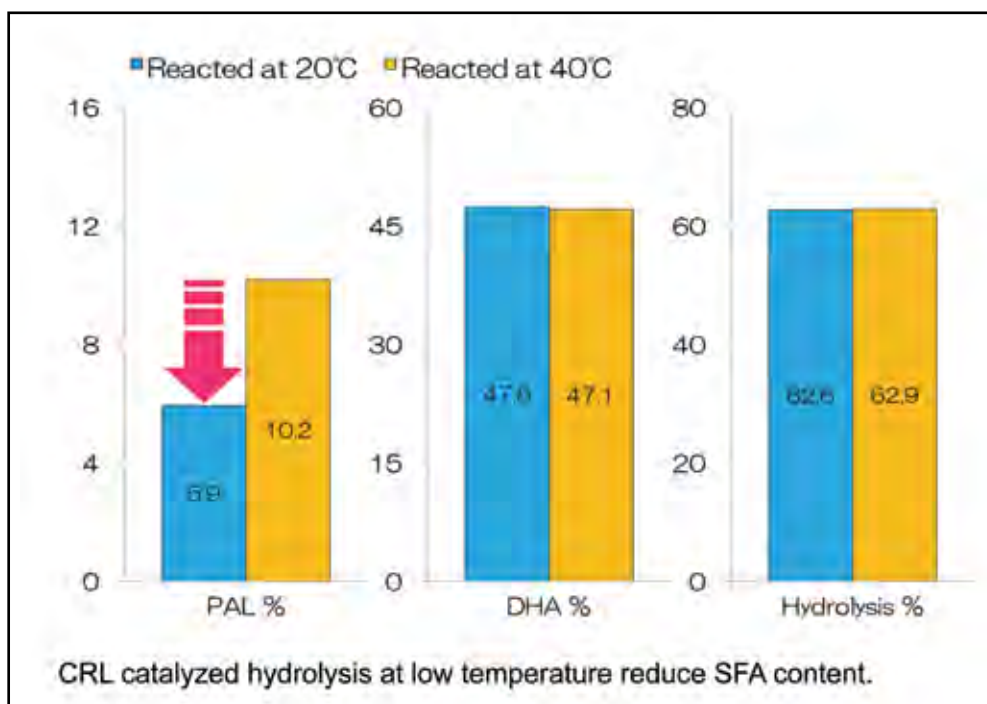


FIG. 3. *Candida rugosa* lipase-catalyzed hydrolysis at low temperature reduces the amount of SFA, but does not affect the rate of DHA content in the glyceride fraction and hydrolysis.

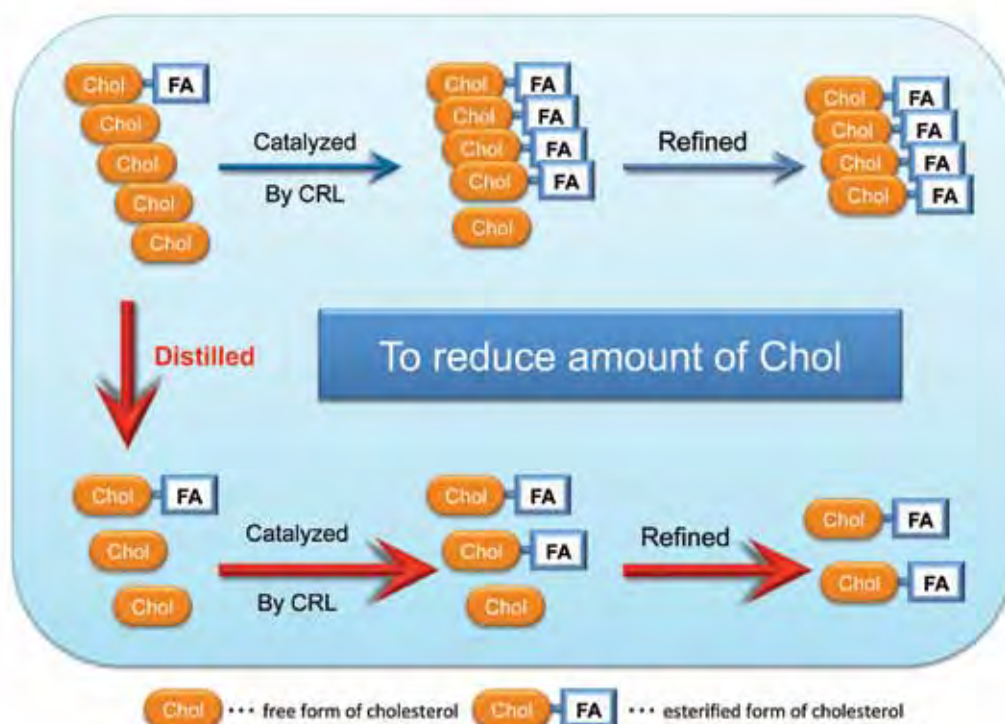


FIG. 4. Esterified form of cholesterol is generated during *Candida rugosa* lipase-catalyzed reaction and is more difficult to remove from oil components. Reducing the amount of free-form cholesterol before catalyzation by lipase results in a relative decrease of cholesterol content in the DHA-enriched oil.

The most unique property of CRL is that TAG is the dominant component in the glyceride fraction, which consists of monoacylglycerol (MAG), diacylglycerol (DAG), and TAG, despite over 50% hydrolysis of the oil [1]. Generally, DAG and MAG are accumulated in the reaction mixture when TAG hydrolysis is catalyzed by lipase.

Hydrolysis is not the only reaction that occurs during CRL catalyzation. Esterification and interesterification also occur, and result in di-PUFA TAG and/or tri-PUFA TAG (Fig. 2, page 62). The rate at which PUFA-containing glycerides are produced by hydrolysis depends on the number of PUFA: mono-PUFA TAG > di-PUFA TAG > tri-PUFA TAG. Hydrolysis kinetics of non-PUFA glycerides is faster than that of PUFA-containing glycerides. Therefore, DHA-rich and TAG-rich oil is obtained.

LOWER TEMPERATURE, LOWER SATURATED FATTY ACID CONTENT

Generally, the optimal temperature of a lipase-catalyzed reaction is approximately 40°C. CRL shows a unique property when the reaction temperature drops. Hydrolysis catalyzed by CRL at around 20°C results in lower contents of saturated fatty acids (SFA) in the glyceride fraction compared with those at 40°C, even though there is no difference in the rate of hydrolysis and DHA content in the glyceride fraction [2]. In addition, TAG content in the glyceride fraction is slightly higher at 20°C than that at 40°C. For example, when the hydrolysis of tuna oil that contains 23.6% DHA and 17.4% palmitic acid (PAL) was catalyzed by CRL at 20°C or 40°C, DHA was concentrated to more than 47% at either temperature, but the content of PAL was 5.9% and 10.2%, at 20°C and 40°C, respectively (Fig. 3, page 63).

Although SFAs play an important role as a source of calories, the modern SFA-rich diet often results in excessive calorie intake. The co-ingested SFA is preferably as low as possible when omega-3 PUFAs are ingested. This technique is suitable for manufacturing health foods or supplements comprising PUFA-rich oils.

REDUCING CHOLESTEROL

Animal oils, including fish oils, generally contain various amounts of cholesterol (Chol). There are two forms of Chol: free and esterified. The esterified form, which binds with fatty acid, is more difficult to remove from oil components than the free form. It is important to reduce the amount of free form before conducting the CRL catalyzed reaction, since the esterified form is generated during the reaction.

Distillation is an efficient way to reduce the amount of free-form Chol. Due to a molecular weight (MW) difference between free-form Chol (MW: 386.7) and TAG (MW: approximately 900), short path distillation can be used to separate Chol from the oil component. This method results in a relative decrease in Chol content compared with the content that is obtained without distillation pretreatment. For example, when tuna oil that contains 0.34% Chol was catalyzed by CRL, the Chol content was 0.48% after refining (Fig. 4, page 63). On the other hand, when distillation was carried out before the CLR-catalyzed reaction, the Chol content decreased to 0.05%—and the amount of Chol remained lower level after CRL catalyzed reaction and refining [3].

NOT EASY TO ESTERIFY GLYCEROL/DHA

Using CRL to synthesize glycerides enzymatically from glycerol and DHA is not efficient. When TAG is hydrolyzed by CRL, DHA is enriched due to the weakness of acting on DHA. Incorporating DHA into the glycerol moiety by CRL-catalyzed esterification is difficult due to the weak action on DHA.

Although other lipases from *Penicillium*, *Pseudomonas* or *Rhizomucor* have been proposed for synthesizing glycerides from glycerol and DHA [4], they don't have enough specificity toward DHA to incorporate it into the glycerol. For these organisms to synthesize DHA-enriched TAG, the DHA must be purified before incorporating it into the glycerol moiety.

Hirouchi Yuji and Doisaki Nobushige are researchers at Nippon Suisan Kaisha, Ltd. They work on processing fish oils, microbial oils or functional lipids, especially concentrating PUFAs and refining oils. Hirouchi's email address is yuuji_hirouchi@nissui.co.jp

Further reading

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