DOI 10.1002/lite.201500036

Feature

Biomimicry – An approach to engineering oils into solid fats

Tzu-Min Wang, Michael A. Rogers

T.-M.W. is a PhD. student at Rutgers in the Department of Food Science, New Brunswick, NJ, 08901.
M.A.R. is an Associate professor at the University of Guelph, Department of Food Science, Guelph, ON, N1G2W1,
E-mail: mroger09@uoguelph.ca.

Summary

The ability to eliminate trans fats, without incorporating additional saturated fats, is limited by the physico-chemical properties of the processed food and what role the lipids play in the food structure. To maintain the levels of cardio-protective unsaturated fats alternative methods to structure them are desperately needed. One such strategy is to utilize oleogels or molecular gels comprised of small molecules. Herein, we illustrate the potential of biomimicking the assemblies formed by the intercellular lipids in stratum corneum using stearic acid, ceramide III and replacing cholesterol with β-sitosterol.

Introduction

Diets elevated in *trans*- and saturated fats have several detrimental health consequences ranging from adverse effects on lipoprotein (cholesterol) profiles, increased prevalence of heart disease and metabolic syndrome. Governments across the globe, have responded by removing the generally recognized as safe (GRAS) status for *trans* fats and are passing aggressive legislation to limit, and in some cases, are even banning the application of *trans* fats, as will be the case in the United States by 2018 [1]. These new legislative policies necessitate the food industry to substitute certain hardstock fats, including partly hydrogenated fats; unfortunately the only current alternative to structuring with *trans* fats is substituting them with saturated fats, which are often negatively viewed by the consumer. As such, alternatives to traditional triacylglyceride (TAG) structuring must be vigorously pursued.

Over the past decade, non-traditional approaches to structuring liquid edible oils into solid fats without the incorporation of other hardstock fats or processing that produces hardstock fats, such as hydrogenation, have been widely investigated, albeit are likely decades away from entering the food supply. Unlike structuring water in foods, gelation of oils is much more difficult due to the poor solubility of food grade polymers. Currently, the only reported food biopolymer capable of gelling vegetable oils is ethyl cellulose [2]. As such, research has focused mainly on the application of low molecular mass oleogelators, which assemble into three-dimensional networks, entraining the liquid oil. These structuring networks may be comprised of liquid crystals, fibrillar crystalline aggregates, platelets, and even colloidal networks similar to traditional fat crystal networks [3]. In oleogels, molecules self-assemble via highly specific, non-covalent interactions that result in the formation of elongated fibrillar structures resulting in the formation of an entangled self-assembled networks or a SAFiN. Forces governing the assembly of the small molecules into more complex aggregates, with long-range order, are poorly understood making the rational design of these systems outside the current realm of possibility. Determining gelation by small molecules is still an empirical science, and the vast majority of new gelators are unfortunately discovered serendipitously [4]. The intermolecular, highly specific interactions that promote preferential 1-dimensional growth are typically hydrogen bonding, pi–pi stacking, electrostatic interactions, van der Waals interactions or some combination thereof. The consequent assemblies formed play the same role as polymer chains in polymeric gels.

In the quest for a hardstock substitute, hydroxylated fatty acids, sorbitol derivatives, combinations of phytosterols and γ-oryzanols, lecithin, and waxes have provided the primary thrust for researchers. With these systems, numerous limitations exist which preclude them from actual edible applications. However, of the aforementioned molecular gelators, waxes are receiving the most attention due to their GRAS status and inexpensive cost, yet their functional properties are not always ideal for food-based applications.

From serendipity to biomimicry

In an attempt to identify new systems capable of structuring edible oils, without relying solely on serendipitous discoveries, we turned our attention to biomimicry. Nature has a remarkable, precise ability to organize small molecules to form extremely complex supramolecular architectures and it has become an active area of materials research ranging from tissue engineering to synthetic bone matrices to ceramics. It was initially our intention to find and exploit a naturally occurring self-assembling system to potentially replace some or all of the hardstock fats in processed foods. This approach made the intercellular lipids in the stratum corneum an interesting starting point due to the assemblies formed comprised of ceramides, free fatty acids, and cholesterol – all of which are naturally present in foods. Aggregates of ceramides, free fatty acids and cholesterol prevent desiccation of the skin by self-assembling, impeding molecular diffusion, which is central to creating an ideal oleogel.

For these novel structures, some of which are capable of structuring edible oils into solid fat oleogels, are comprised of the bioactive lipids found the mammalian epidermis (i.e., the ceramides, fatty acids and cholesterol (which will be replaced in our study with plant phytosterols). With so many classes of known molecular gelators, why focus on combinations of ceramides, phytosterols and fatty acids? Each of the classes of compounds are good target orga-

nogelators because they are sourced from common food ingredients, each are commercially available with different chemical structures and a vast body of work exists which has indirectly studied their assembly in skin. What is truly remarkable about this system is beyond their ability to eliminate the heart unhealthy *trans* fats without drastically increasing the saturated fats AND while maintaining the viscoelastic properties of solid fat is that both ceramides and phytosterols have numerous beneficial health effects. For example ceramides are referred to as tumor suppressing lipids. Ceramides promote the production of interlukin 1B, which causes apoptosis in tumor cells. There are numerous possible health implications for the development of a ceramide based spreadable fat product. Possibly, the most exciting is the role of ceramides as inhibitors of colon carcinogenesis since ceramides alter cell growth, differentiation, and apoptosis. Similar to ceramides, β-sitosterol has both anti-inflammatory and cholesterol lowering effects. Specific mixtures of ceramides, fatty acids and β-sitosterol, dispersed in vegetable oil, produce edible organogels that are food-grade and mimic the physical properties of margarine with NO *trans* fats and less than 5% added saturated fat to oils such as canola and corn oils!

Individually each are poor gelators

Pure short chain ceramides may be used as a strategy to structure apolar liquids into solid-like plastic materials. Early investigations appear to suggest that increasing the chain length modifies the crystal structure from a fibril aggregate to small spherulites or platelets [5]. Mixed chain length and long chain length ceramides are less effective gelators. β-sitosterol, long chain ceramides (Ceramide III) nor stearic acid are effective at forming solid fats in canola oil. At 5 wt% stearic acid and ceramide III forms platelets, while β-sitosterol forms spherulites (**Figure 1**).

Mixing the structurants

When mixing β-sitosterol and fatty acids, in some instances, the crystals transition from platelets to ribbons, which is truly remarkable (**Figure 2**)! When the stearic acid and β-sitosterol is mixed in a 4 : 1 ratio the aspect ratio of the crystals is approximately 2 : 1. When the ratio is modified from 4 : 1 to 2 : 3 we see a drastic increase in the aspect ratio of the crystals (5 : 1). Further decreasing the stearic acid to a ratio of 1 : 4 no longer has a significant effect on the crystal morphology. Although the application of cholesterol is unlikely to be a novel fat substitute, the effects of mixing stearic acid and cholesterol on the crystal morphology is even more pronounce compared to β-sitosterol. Similar changes are observed regardless of if stearic acid is mixed with either cholesterol or β-sitosterol at 4 : 1 or 2 : 3 ratios. However, with stearic acid and cholesterol when the ratio is 1 : 4 the aspect ratio drastically increases (~1000 : 1). Although these fibers are likely not appropriate in foods because they are often undesirably detected in the palate they may have numerous other applications in structuring cosmetics and delivery pharmaceutics.

As the system complexity increases and a second gelator is added, the supramolecular properties vary greatly. A ternary phase diagram was established using the three structurants totaling 8 wt% and were varied at 1 wt% intervals in canola oil (**Figure 3**). All twenty-one ratios had drastically different crystal morphologies while only 8 were capable of structuring the liquid oil into solid-like matrices. Meaning that these samples did not flow upon vial tube inversion. Samples that were comprised of less than 3 wt%

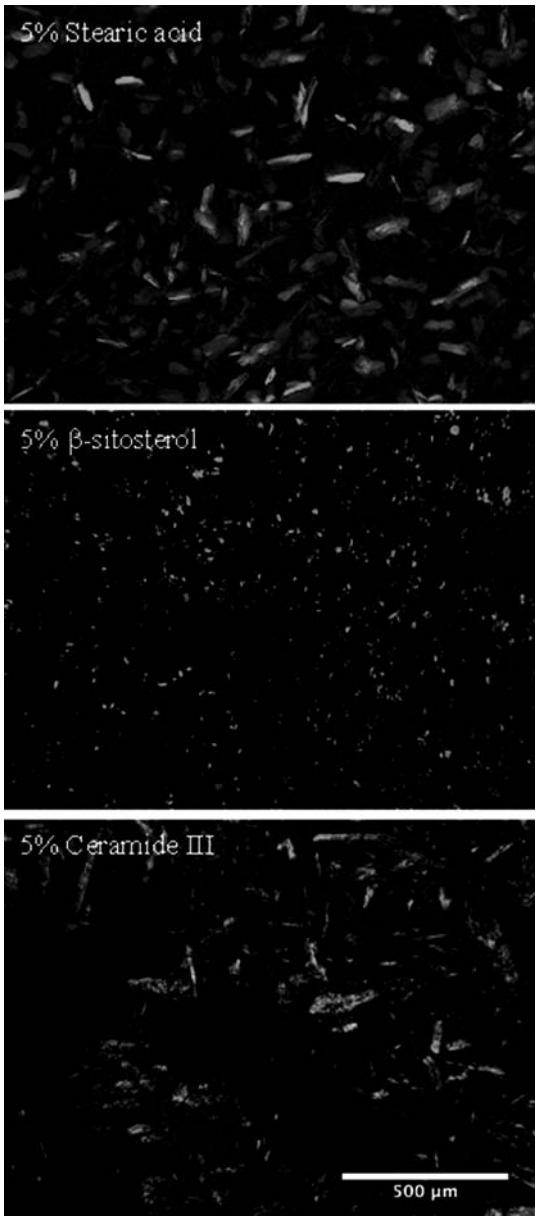

Figure 1. Polarized light micrographs of viscous solutions of 5 wt% stearic acid, β-sitosterol and ceramide III in canola oil.

stearic acid tended to flow when inverted and had a G″ greater than G′ with the exception of (2 wt% stearic acid, 1 wt% β-sitosterol and 5 wt% ceramide III (SBC215) and SBC 314. Fibers were observed at five of the ratios (SBC314), SBC413, SBC512, SBC521 and SBC611), while platelet-like crystals (SBC215), short fibril crystals (SBC422), and rosette-like crystals (SBC431) were observed in the remaining solid-like samples. Three of the samples that appeared to be gels using the vial inversion technique were not actually gels (SBC215, SBC422, SBC431) upon further investigation. When observing the frequency dependence of the samples comprised of platelets, short fibers or rosette-like crystals they were not true gels because they exhibited frequency dependence of G′ and G″.

The five specific ratios that formed crystal fibers each exhibited similar compositions (i.e., β-sitosterol concentration was either 1 or 2 wt% (i.e., on the right side of ternary phase diagram (Figure 3)). The mixed ratios (SBC314, SBC413, SBC512, SBC521 and SBC611) that consisted of fibers each had an elastic modulus (G′)

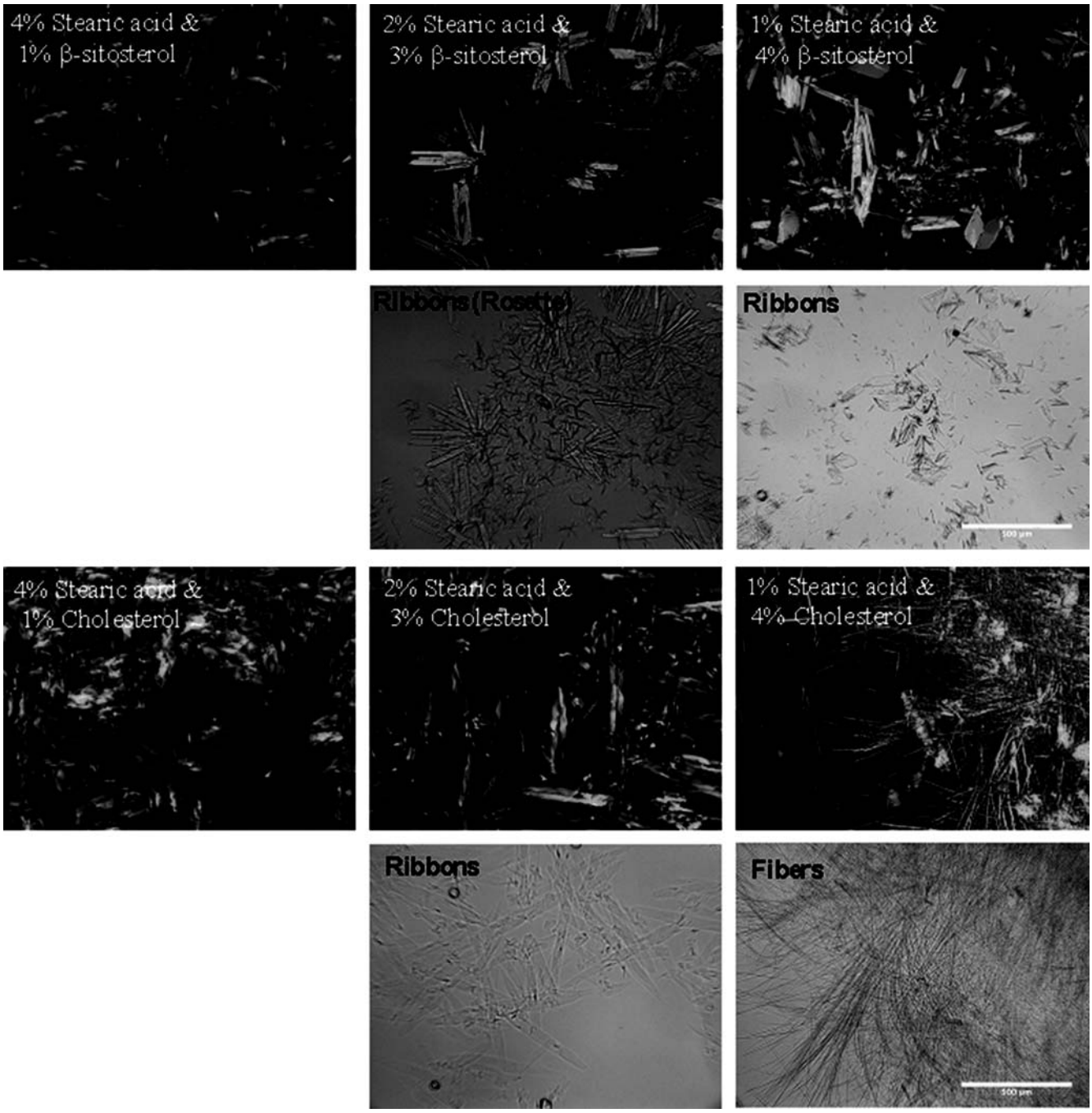

Figure 2. Polarized light micrographs (top) and brightfield micrographs (bottom) of 5 wt% combinations of stearic acid and β-sitosterol as well as stearic acid and cholesterol in canola oil.

greater than the loss modulus (G″) and were frequency independent between 0.1 and 10 Hz. The elastic modulus ranged from 100 Pa to 10^5 Pa at 8 wt% depending on the ratio of the three structurants. This facilitates a great range of tailoribility depending on the end application. At 100 Pa the materials is a viscous oil that flows and may have applicability in sauces while the materials that have a storage modulus 10^5 Pa could be used as margarine substitutes. The yield stress of SBC611, SBC521 and SBC512 were 4.65 ± 2.36, 1.22 ± 0.73 and 3.43 ± 2.07 Pa; respectively, indicating they are easily spread.

Not only can the hardness of the gels be tailored, using different structurant compositions, but so could their melting properties. The onset of melting was between 42 and 48 °C and the onset of crystallization was between 19 and 29 °C. For the solid samples, when a greater concentration of stearic acid compared to β-sitosterol the sample had a higher melting and crystallization temperature.

Conclusions

The ability to eliminate *trans* fats, while limiting saturated fats, while maintaining the physic-chemical properties of the food is a daunting task that is in dire need of novel technologies. One strategy is to utilize oleogels or molecular gels comprised of small molecules. The oleogels derived from stearic acid, ceramide III and β-sitosterol to structure edible oils has tremendous potential to create a new solid fat with a healthier profile.

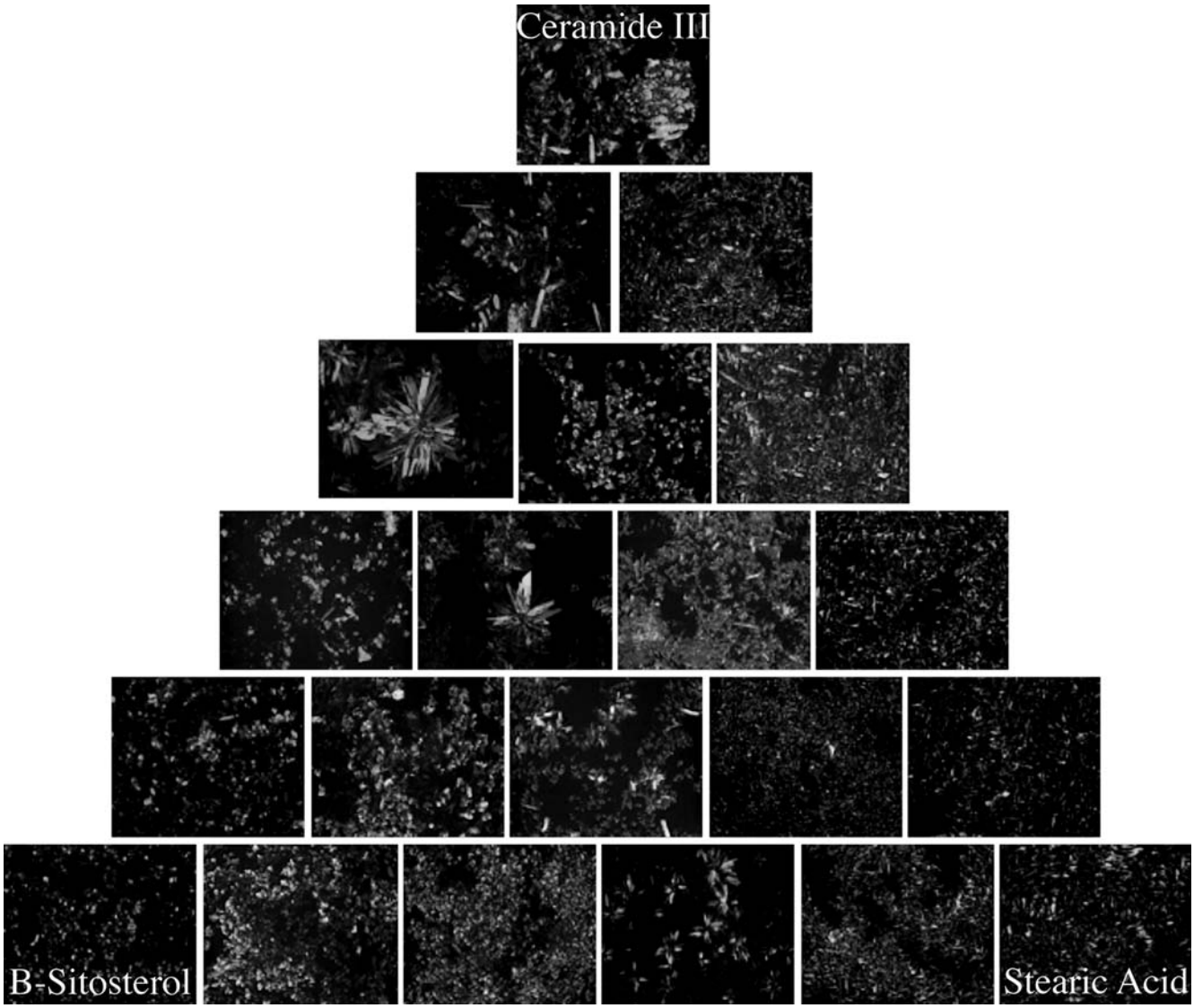

Figure 3. Ternary phase diagram consisting microscopy images for twenty-one samples. The composition of each point is expressed as the stearic acid: β-sitosterol: ceramide III ratios in canola oil.

References

[1] FDA, U.S. Food and Drug Administration, 2015, vol. *2015*.
[2] Gravelle, A.J., Barbut, S., Marangoni, A.G., *Food Research International* 2012, *2*, 578–583.
[3] Rogers, M.A., *Food Research International* 2009, *42*, 747–753.
[4] Lan, Y., Corradini, M.G., Weiss, R.G., Raghavan, S.R., Rogers, M.A., *Chem. Soc. Rev.* 2015, DOI: 10.1039/C5CS00136F.
[5] Rogers, M.A., Wright, A.J. Marangoni, A.G., *Soft Matter* 2009, *5*, 1594–1596.