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Mechanical Response of Single Triacylglycerol Spherulites by Using Microcolloidal Probes

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First insights into the mechanical properties of a single fat spherulite were obtained by the indentation with microcolloidal probes. We found that the single spherulite of triacylglycerol acts like an elastic body after pushing several times with a microcolloidal probe. The calculated elastic modulus for the single spherulite was comparable with that of condensed triacylglycerol samples, measured using a bulk rheometer, despite an apparent difference in morphology and volume fraction.

Keywords: Fat spherulite | Mechanical property | AFM force measurement

Macroscopic mechanical properties of fat crystal networks have attracted much attention in soft matter physics, although they have been studied for more than four decades.[1] The majority of fat products available to the consumer like margarine and ice cream are structured by hierarchical networks of triacylglycerol (TAG) crystals, which can stabilize food emulsions and thus have a significant impact on taste and texture. On the other hand, there has been an increasing social demand to reduce man-made *trans* and saturated fatty acids from food products because ample evidence suggests that they cause deleterious health conditions, such as atherosclerosis, heart disease, and metabolic syndrome.[2–5] Although simply eliminating these unhealthy fats seems one solution, it also causes the loss of solid structure, which has an implication for the mechanical properties of fat-based food materials. Thus, in order to explore an alternative, it is important to understand how the microscopic structural order of fat crystals is correlated with their macroscopic mechanical properties.[6,7] Actually, the importance of the hierarchical structures of fat crystal networks has been recognized for over 50 years.[7–13] Techniques like infrared spectroscopy, nuclear magnetic resonance, differential scanning calorimetry, and X-ray diffraction/scattering have been used to identify and characterize these structural levels.[14–17] Recent innovations in cryo-TEM techniques have succeeded in identifying highly anisotropic primary crystals, crystalline nanoplatelets (CNPs) of TAG polycrystalline networks.[18,19] CNPs consist of TAG multilayer stacks with thickness of a single TAG layer being 3–5 nm. X-ray experiments using ultrasmall-angle X-ray scattering (USAXS) has also identified primary crystallites that were similar in size to those observed under Cryo-TEM.[20,21] CNPs aggregate into micrometer-sized particles of different morphologies including spherulites. Spherulites have a central core of tightly packed crystals surrounded by radially oriented needles. Polycrystalline networks structured with spherulites are ubiquitous in many food materials, including starch and fat, and play an important role in defining the macroscopic rheological properties, and thus texture, of food.[22] In this work, we present the first insights into the mechanical properties of a single fat spherulite by the indentation technique using atomic force microscopy (AFM). Since the surfaces of spherulites are not molecularly smooth, a microparticle-attached cantilever (microcolloidal probe) was used to avoid the indentation of free voids. This enables one to utilize a large contact area of the spherulite sample, which cannot be achieved by conventional cantilevers with pyramidal tips with typical radii of curvature $R_c \approx 10$ nm.[23]

Fully hydrogenated canola oil (FHCO) and liquid high oleic sunflower oil (HOSO) were generously provided by Bunge Canada (Toronto, Canada) and Nealanders (Toronto, Canada), respectively. Blends of FHCO and HOSO were prepared in 3:7 ratios and subsequently held at 80 °C for 30 min to erase crystal memory. Then, the samples were statically crystallized at 20 °C in order to promote the β polymorphic (triclinic) form. X-ray diffraction patterns collected at 20 °C confirmed the presence of the desirable polymorphism. Finally, blends were kept at 20 °C and diluted with HOSO to the required consistency prior to analysis. Mechanical properties of single spherulites were characterized by the indentation measurements with an AFM (NanoWizard, JPK Instruments, Berlin, Germany). A silica microparticle (diameter: 5 μm), was glued on a cantilever (spring constant of 0.01–1.0 N m^{-1}, Brucker, Mannheim, Germany) with water-insoluble epoxy (Endfest 300, UHU, Germany).

Figure 1 shows representative bright field (BF) and polarized light microscope (PLM) images of TAG spherulites from the condensed mixture. The intent of the images was to exhibit the characteristic length scales of the spherulites and their constituents rather than being an introduction into a more extensive study using BF and PLM. The typical diameter of spherulites was 10–50 μm depending on the mixture ratio between FHCO and HOSO. As presented in Figures 2a and 2b, a single spherulite under a highly diluted condition (crystal (FHCO):oil (HOSO) (w/w) = 3:10000) was pushed and retracted 8 times by a microcolloidal probe. Figure 2c shows the force curves from the first push (black) and retraction (red) cycle. The force dropped several times during pushing, while the retraction curve was apparently very smooth. The global shape of the retraction curve determines the mechanical properties of spherulites using the Hertz model.

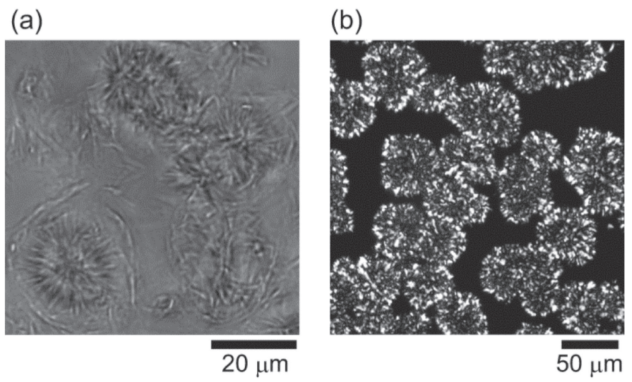

Figure 1. (a) BF and (b) PLM images of FHCO/HOSO TAG spherulites. The mixture ratios between FHCO and HOSO were (a) 3:97 and (b) 3:7.

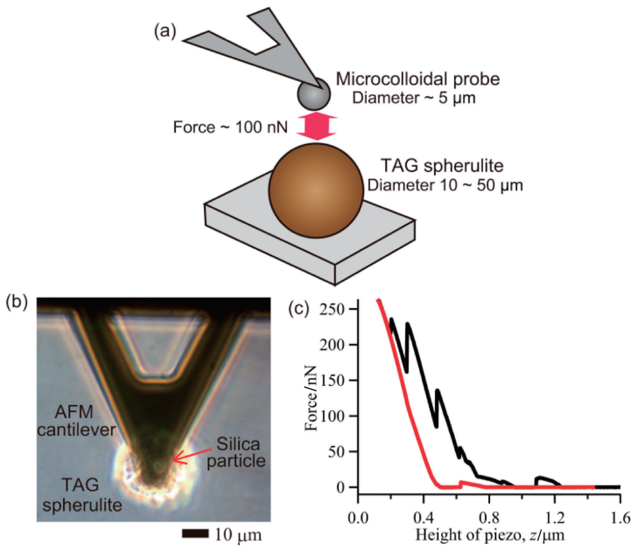

Figure 2. Force measurement of a single TAG spherulite with a microcolloidal probe. (a) A schematic view of the experimental setup. (b) A BF image of the force measurement. (c) Force–distance curves of a single TAG spherulite pushed (black) and retracted (red) with a microcolloidal probe.

Figure 3a shows the force–indentation curves of a single spherulite under repetitive push/retraction cycles. The force curves showed a monotonic decrease in the number of sawtooth-like profiles, except for the 4th push curve. After the 5th push, the global shape of curves seemed almost identical, showing no abrupt drops. Here we focus on the quantification of a specific mechanical property of the spherulites, the Young's modulus E_{fat}. The variable was calculated from the analysis of the measured force curves with the Hertz model assuming the contact between two spheres:[24]

$$F = \left(\frac{4}{3}\right) E^* \sqrt{\left(\frac{1}{R_{fat}} + \frac{1}{R_{probe}}\right)} \delta^{3/2} \qquad (1)$$

where F is the force and δ is the indentation depth given by subtracting the cantilever deflection $d(z)$ from the piezo movement $z_0 - z$, z_0 being the height of contact point. R_{fat} and R_{probe} are the radii of a spherulite and a silica microparticle,

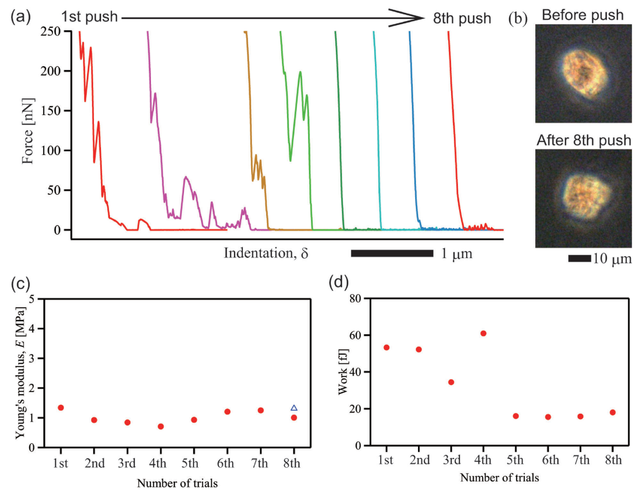

Figure 3. Mechanical characterizaition of a single spherulite under repetitive push/retraction cycles. (a) Force–indentation curves obtained from 1st to 8th indentation. (b) BF images of the single spherulite before the indentation measurement (upper) and after 8th indentation (lower). (c) Young's moduli of the single spherulite obtained by the fitting of each force curve for the region of 215 nN < F < 250 nN (red circle) or ca. 0 nN (point of contact) < F < 250 nN (blue triangle, 8th push only). (d) Work necessary to reach $F \approx 250$ nN. The work was calculated by the integration of each force curve.

respectively. The global radius of a fat spherulite (R_{fat}) determined from BF images was kept constant throughout the analysis because the size of spherulites did not change significantly before and after several indentation cycles (Figure 3b). It should be noted that we ignored the deformation of the underlying substrate because spherulites were deposited on a hard polystyrene substrate ($E \approx$ GPa). E^* is the effective elastic modulus given by

$$E^* = \left(\frac{1 - \mu_{fat}^2}{E_{fat}} + \frac{1 - \mu_{probe}^2}{E_{probe}}\right) \qquad (2)$$

where μ_{fat} and μ_{probe} are the Poisson's ratios of spherulites and microparticles, which are assumed to be 0.5 and 0.25, respectively.[25] Since the Young's modulus of a silica microparticle (≥10 GPa) is about 3 orders of magnitude larger than E_{fat}, the latter term, $(1 - \mu_{probe}^2)/E_{probe}$, in eq 2 was ignored in the analysis.

Figure 3c shows the Young's modulus calculated from each force curve. For the quantitative comparison, we fitted the force curves within the large force region (215 nN < F < 250 nN) that exhibited no force drops (Figure S1). We found that the Young's moduli obtained were almost constant (ca. 1 MPa) for all the indentation curves (1st to 8th), implying that the core of the measured spherulite acts like a linear elastic body. We also calculated the work U required while performing the indentation by integrating each force curve ($F \geq 0$ N). The work necessary to reach $F \approx 250$ nN was $U \approx 50$ fJ in the first two measurements. It then decreased to a saturation level $U \approx 15$ fJ for the latter four measurements.

These results indicate that a spherulite was annealed after several indentation cycles and finally acted like a simple elastic sphere. In fact, we found that a whole region ($F < 250$ nN) of the force curves could be well-fitted by the Hertz model, once the force curve reached saturation. We found a similar tendency for the other spherulites on the same substrate; force curves become smooth after some pushing/retracting cycles. Then we calculated E_{fat} of each single spherulite from the force curves that showed no force drops after the push/retraction cycles (Figure S2). The averaged E_{fat} obtained by the fitting from $F \approx 0\,\mathrm{N}$ (a point of contact) to $F \approx 250\,\mathrm{nN}$ were $1.9 \pm 1.0\,\mathrm{MPa}$ (FHCO:HOSO (w/w) = 3:10000, $n = 5$) and $4.0 \pm 1.0\,\mathrm{MPa}$ (FHCO:HOSO (w/w) = 3:1000, $n = 6$). Interestingly, the E_{fat} obtained for single spherulites from the highly diluted condition was comparable to the averaged storage modulus of $G' \approx 1.9\,\mathrm{MPa}$ for the condensed TAG sample (FHCO:HOSO (w/w) = 3:7) measured with a conventional rheometer.[26] This finding that spherulites possess mechanical toughness (bulk elastic modulus) comparable with that of bulk fat materials suggests the critical role of networks of small crystallites in maintaining the stability of food colloids. The energy ($U \approx 15\,\mathrm{fJ}$) obtained from the 5th to the 8th indentation curves (Figure 3d) is concerned with the deformation of a single spherulite, involving the viscoelastic responses of the solid structures and liquid oil inside spherulite (Figure S3). On the other hand, the additional work $\Delta U = 20$–$45\,\mathrm{fJ}$ dissipated by the 1st to 4th indentations seems to coincide with the reordering (annealing), and thus healing, of defects without a major alteration of the global radius. The observed relaxation might be attributed to the reordering of "TAGwoods",[21,27] whose characteristic length scales, $d = 0.2$–$1.5\,\mu\mathrm{m}$, were determined by ultrasmall-angle X-ray scattering.[27] Once spherulites have been mechanically "annealed" by the repetitive pushing cycles, they behave like linear elastic spheres, which qualitatively agree well with the spontaneous relaxation predicted from the theoretical model.[20] It should be noted that such discrete mechanical responses of single spherulites cannot be observed in the macroscale using a conventional rheometer but can only be revealed by microscopic AFM indentation. Further experiments using PLM at high resolution with a retardation plate may reveal how the spherulites' constituents rearrange when probed by the cantilever.

In summary, we have quantitatively evaluated the mechanical properties of single fat spherulite of FHCO in HOSO by repetitive indentation measurements. The force curves measured by microparticle-attached cantilevers reflect the hierarchical mechanics of fat spherulites; therefore, they bridge the gap between fine-structural characterization (e.g. X-ray scattering and electron microscopy) and bulk rheology. This is the first measurement of the elastic properties of fat spherulites in the extreme strong link regime and not attainable by bulk rheological measurements. They provide information on the micromechanical characterization of essentially *non-interacting* fats particles as compared to bulk rheology, which deals with higher concentrations (e.g., bulk with the ratio of solid oil:liquid oil ≈ 10:1), and likely *interacting*, fat particles. The characteristics of fat crystal networks have major impacts on the functional properties of bulk fat, in terms of rheological, thermal, and sensory properties. Thus, we foresee that mechanical properties of spherulites can be used as quantitative indicators toward the engineering of the functionality of fats and fat-structured food materials.

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Supporting Information is available on http://dx.doi.org/10.1246/cl.170014.

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