Heterogeneous Nucleation of 1,3-Distearoyl-2-oleoylglycerol on Tristearin Surfaces

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ABSTRACT: The following work investigates the heterogeneous nucleation of 2-oleodistearin (SOS) triglycerides on surfaces formed by crystals of tristearin triglyceride (SSS). This work shows, through computer simulations and nucleation kinetics, that SOS may heterogeneously nucleate on SSS surfaces. Atomic-scale molecular dynamics showed that SOS molecules exhibited an affinity to a simulated SSS surface. Nucleation kinetics using differential scanning calorimetry showed that the inclusion of minor amounts of SSS (from 1 to 4%) in an SOS melt resulted in an increase in the isothermal nucleation rate of crystallizing SOS. Using a model based on the Fisher–Turnbull approach, estimates of the surface free energy, activation free energy, and the critical radius were calculated from the nucleation rates. The estimated parameters demonstrate the heterogeneous nucleation of SOS on SSS surfaces: reduced surface free energies, activation free energies, and critical radii with the inclusion of SSS in an SOS melt. This may point to strategies to enhance the nucleation of one of the three major triglycerides present in cocoa butter and the one that crystallizes first from the melt for better control of the chocolate tempering process.

INTRODUCTION

Cocoa butter is a premium fat used in the manufacture of confectionery products such as chocolate bars and enrobed confectionery products.1 It consists predominantly of three triglycerides: 2-oleodistearin (SOS), 2-oleodipalmitin (POP), and 1-palmito-2-oleo-stearin (POS). These 2-oleo-triglycerides collectively account for about 90% of the triglycerides in cocoa butter.2 It is generally agreed that cocoa butter can crystallize into six different crystal forms (form I to form VI), which are subtypes of the three polymorphic forms (α, β′, and β) into which fats commonly crystallize into.3 Of the six forms, form V is the most desirable polymorphic form as it results in optimal textural and visual characteristics for chocolate.

To achieve the desired polymorphic form, chocolate mass must be processed using a controlled temperature protocol known as tempering. Achieving the desired polymorphic form can also be attained by seeding the chocolate mix. Seeding is the addition of the preformed crystalline material (called seeds) of the desired polymorphic form to facilitate crystallization into the same polymorphic form as well as to accelerate crystallization. It is commonly agreed that seeding facilitates crystallization through a process called “secondary nucleation” although an exact description of this phenomena remains elusive.4 The seed material is commonly cocoa butter although novel fats chemically dissimilar to cocoa butter have also been used.5

Cocoa butter is an expensive fat. Due to a combination of environmental, economic, and political factors, the price of cocoa (from which cocoa butter is derived) has surged considerably between the years 2013 to 2016, from approximately United States $2.15/kg (March 2013) to $3.12/kg (June 2016). The price has since decreased to $2/kg (Sept 2017) but has risen to $2.66/kg as of May 2018.6 The volatility in cocoa prices as well as the vulnerability of its supply has spurred the development of cocoa butter equivalents (CBEs), fats that are chemically similar to cocoa butter. CBEs are considered superior to cocoa butter replacers (fats with similar functionality as cocoa butter but dissimilar chemical compositions such as palm kernel oil) in that they can be blended with cocoa butter. In theory, cocoa butter equivalents can be formulated by blending purified shea stearin (rich in SOS) with purified palm mid-fraction (rich in POP). Other CBEs can be formulated from single-cell sources such as algal butters.7

An important consideration in the formulation and use of CBEs is the inevitable inclusion of minor components in the fat. Total removal of these impurities would reduce or eliminate the economic incentive to use CBEs. One such component is the trisaturated triglyceride tristearin (SSS). Tristearin is naturally present in extracted cocoa butter at mass percentages between 0.2 and 1.0% wt/wt, with the total amount of trisaturated triglycerides typically between 2.0 and...
The impact of tristearin on the performance and functionality of CBEs (as well as cocoa butter) is an important consideration, which has merited considerable research attention.

Trisaturated triglycerides such as SSS have higher melting points than their corresponding sn-2-oleic acid triglycerides such as SOS. The phase behavior of a simple binary mixture of SSS and SOS was shown to be monotectic in that a single solid phase (with a melting point intermediate between the two binary components) was formed. In the analogous palmitic acid system (PPP/POP), the same monotectic behavior was observed. In the solid phase formed by a mixture of SSS/SOS, the 2-oleodiestearin was shown to be more soluble in the tristearin, with a tristearin-majority phase being able to take in up to about 50% SOS. SSS is not at all soluble in SOS—the amount of SSS being taken into the solid solution by the SOS-majority solid phase was very small, well below the resolution of the phase diagram. Similar behavior was observed for the PPP/POP system. Therefore, in an SSS/SOS mixture with a significant amount (>1–2%) of SSS, SSS can be expected to crystallize into a solid phase separate from the SOS solid phase, prior to the crystallization of SOS. This is evidenced as a clouding of cocoa butter at the start of cocoa butter crystallization.

The impact of the crystallization of the SSS fraction on the crystallization of SOS is the subject of a long-running debate. Because SSS crystallizes prior to the rest of the cocoa butter, there is considerable interest in whether or not SSS can act as an indigenous seeding material in cocoa butter. According to Talbot, the significance of SSS in the crystallization of cocoa butter is not in its ability to seed the crystallizing material but in its ability to increase the viscosity of the crystallizing cocoa butter and thus create an impediment during processing, such as in mold-filling and enrobing.

Furthermore, according to Smith, trisaturated triglycerides cannot act as seeds for the subsequent crystallization of sn-2-oleic acid triglycerides (such as SOS) as the crystalline structure of trisaturated triglycerides (2L) is different from that of the sn-2-oleic acid triglycerides (3L). Triglycerides crystallize by stacking in layers called lamellae. The thickness of these lamellae is dependent on the triglycerides that make up these lamellae. Trisaturated triglycerides stack in crystalline lamellae with thicknesses that are twice the chain-lengths of the constituent fatty acids (hence 2L) as saturated fatty acid chains are generally complementary to other fatty acid chains provided the chain length difference is not too great (more than four carbons). However, sn-2-oleic acid triglycerides, because of the kink introduced by the double bond of the sn-2-oleic acid group, stack in crystalline lamellae that are 3 times the length of the fatty acid chains. This minimizes the disruption of the crystal structure introduced by the kink by effectively segregating the oleic acid chains away from the saturated fatty acids attached at the sn-1 and sn-3 positions.

Hachiya and others studied the effect of cocoa butter (in various polymorphs), SOS (in various polymorphs), BOB (2-oleyl dibehenin, in various polymorphs), and SSS (β′ polymorph) and their ability to temper a mass of cocoa butter and dark chocolate. The efficacy of tempering was evaluated by how well the seed material reduced the crystallization time, the time required to reach a certain viscosity (evaluated as the torque in a model scraped-surface heat exchanger). The efficacy of tempering was found to be strongly dependent on the chemical species and the polymorph. The mechanism of tempering was postulated to be secondary nucleation, whereby the seed material (and fragments thereof) acted as nuclei for subsequent crystal growth. SSS (in the β′ polymorph) was shown to reduce the crystallization time but only by 10–20%, depending on the amount of seed material added.

Wähnelt, Meuse, and Tülsner added various diglycerides and tripalmitin to cocoa butter and found that the addition of tripalmitin (albeit at a concentration of 10%) reduced the onset time for crystallization. Loisel and others studied the addition of 0.3, 1.0, and 1.6% SSS to a dark chocolate (naturally containing 3.0% trisaturated triglycerides) crystallized dynamically using a scraped-surface heat exchanger. The dynamics of chocolate crystallization were characterized by the torque changes necessary to maintain a steady rate of rotation of the SSHE shaft. It was observed that the addition of SSS reduced the time between the first and second torque jumps (indicative of the acceleration of the crystallization of SSS). As well, the addition of SSS increased the magnitude of the second torque jump. Furthermore, the authors estimated the amount of SSS that had crystallized and remained in solution. Of the 3% trisaturated triglycerides present in cocoa butter, the authors estimated that, at 28.2 °C (chosen to promote the crystallization of only the β′ form of SOS), approximately 66% (that is, 2%) of the trisaturated triglycerides remained in solution, whereas 33% (that is, 1%) crystallized out of solution.

Campos and others (2009) studied the effect of adding SSS at various levels to cocoa butter. The addition of SSS increased the onset temperature of crystallization from 17.08 to 22.23 °C and reduced the induction time of crystallization from 36 to 22 min. This was attributed to the assumed co-crystallization of the SSS with the higher melting fraction of the cocoa butter. The co-crystallization of the SSS with cocoa butter triacylglycerols (TAGs) was shown to reduce the thickness of the crystalline TAG particles. The addition of SSS also reduced the rate of transformation from the β′ polymorph to the β′ polymorph. The addition of SSS also induced a softening effect (reduction in the elastic bending modulus) in the solidified cocoa butter. This was attributed to the reduction of the crystal-melt interfacial energy (or surface energy) brought about by the introduction of increased disorder due to the co-crystallization of SSS with cocoa butter triglycerides.

The main method for measuring the nucleation rate in crystallizing fats, turbidometry, is inadequate in situations where other suspended particles are present (such as chocolate mass) and in situations where there is more than one crystallizing species (fractional crystallization). This work presents studies on the measurement of nucleation rates in the crystallization of SOS (a cocoa butter TAG) in the presence of SSS. This work employs computer simulations (atomic-scale molecular dynamics, ASMD) and differential scanning calorimetry (DSC) to advance the following thesis: SSS can facilitate heterogeneous nucleation of cocoa butter triglycerides such as SOS. The crystallization of SSS creates planar methyl surfaces to which other TAGs can adsorb. This TAG surface adsorption increases the nucleation rate by reducing the surface energy penalty of the activation free energy of nucleation (Figure 1).

### RESULTS AND DISCUSSION

**Atomic-Scale Molecular Dynamics.** The ASMD results show that in the case of the triglycerides SOS and OOS, the layered state exhibits a lower free energy per molecule than the
mixed state (Figure 2). In the case of OSS and OSO, the error bars (root-mean-square) overlap, indicating no free energy difference between the layered and mixed states. These results suggest that SOS and OOS will exhibit an affinity for an SSS surface, given that the free energy of the layered configuration of these TAGs is lower than that of the corresponding mixed configurations.

The affinity of SOS for the SSS surface supports the hypothesis that SOS may heterogeneously nucleate on the SSS surface. An affinity of SOS for the SSS surface implies a “wetting” of the surface by SOS, which would reduce the activation free energy for nucleation. The free energy for the homogeneous nucleation of a cylindrical nucleus from the melt is the sum of two contributions, a bulk term and a surface term:

\[ \Delta G_n = \Delta G_{\text{surface}} - \Delta G_{\text{bulk}} \]  

\[ \Delta G_{n,\text{ho}} = (2\pi^2 + 2\pi r h)\gamma_{r-1} - (\pi^2 h)\frac{\mu^l - \mu^s}{V_M} \]  

where \( \gamma_{r-1} \) is the interfacial free energy per unit area, \( r \) is the radius of the cylinder, \( h \) is the height of the cylinder, \( V_M \) is the molar volume of the solid, and \( \mu^l \) and \( \mu^s \) are the chemical potentials of the liquid and solid phases, respectively. The chemical potential difference is achieved via the supersaturation of the liquid phase.

Consider the heterogeneous nucleation of a solid cylindrical SOS nucleus on a surface, typically called the condensation nuclei or CN, in homage to its origins in atmospheric phenomena. One face of the cylindrical nucleus (with area \( \pi rh \)) would be in contact with the CN surface (Figure 1). The surface term can be split into two terms and the free energy of heterogeneous nucleation is written as:

\[ \Delta G_{n,\text{he}} = (\pi^2)\gamma_{r-\text{CN}} + (\pi^2 + 2\pi rh)\gamma_{r-1} - (\pi^2 h)\frac{\mu^l - \mu^s}{V_M} \]  

where \( \gamma_{r-\text{CN}} \) is the interfacial free energy between the SOS cylinder face and the CN. If \( \gamma_{r-\text{CN}} \) is less than \( \gamma_{r-1} \) or even zero, then the heterogeneous free energy of nucleation is effectively less than the homogeneous free energy of nucleation for the same cylinder. Heterogeneous nucleation is, in essence, the minimization of the surface between the solid-phase nuclei and the liquid phase melt by “shielding” some of that surface via nucleation on a foreign surface, the CN. For as long as \( \gamma_{r-\text{CN}} \) is lower than \( \gamma_{r-1} \), heterogeneous nucleation is always energetically more favorable than homogeneous nucleation. As such, heterogeneous nucleation exhibits a higher nucleation rate than homogeneous nucleation.

The simulation results can be interpreted as a \( \gamma_{r-\text{CN}} \) that is lower than \( \gamma_{r-1} \). Indeed, since \( \gamma_{r-\text{CN}} \) is the interfacial free energy between two solid phases, it can be assumed to be effectively zero. The simulation shows that SOS and OOS, but not OSS or OSO, exhibit an affinity for the SSS surface as shown by the lower free energy for the layered state for SOS and OOS than for the mixed bulk state for SOS and OOS. The affinity for the surface implies that SOS and OOS will bind to the SSS surface, resulting in a lower surface free energy that will lead to heterogeneous nucleation. An additional argument is that the affinity of the SOS and OOS to the SSS surface “concentrates” these triglycerides on the surface such that the collision...
frequency necessary for nucleation is increased. As will be shown in the next section, the affinity of SOS triglycerides to the SSS surface is manifested as an increase in the nucleation rate of SOS in the presence of an SSS surface.

**Nucleation Kinetics Results. Induction Time Measurements.** The exothermal trace obtained from the isothermal step (step #4) of the temperature program shows a well-defined Gaussian peak (Figure 3). This peak is generated when the material in the pan starts to crystallize. The more asymmetric the peak shapes become, the lower is the supersaturation (i.e., higher isothermal temperature). At sufficiently high temperatures (~28 °C), multiple peaks may sometimes be detected (Figure 4). This is assumed to be due to the crystallization of various polymorphic forms. Due to the additional complexity introduced by polymorphic transformations, the 28 °C temperature point was not included in later free energy calculations via the Fisher–Turnbull method.

For fractionated shea stearin containing approximately 80% SOS by the peak area (Shea Stearin 1), the optimal temperatures for measuring well-spaced induction times are between 23 and 28 °C. The optimal temperature range for obtaining good induction time data is dependent on the amount of crystallizable SOS in the material preparation. This is not unexpected since a preparation containing more SOS achieves the same supersaturation at a higher temperature than a preparation containing less SOS.

Determination of the induction time is subjective. Given that the peak shapes are symmetric Gaussian peaks, a natural measure of the induction time is the onset time, which is the time at the point of intersection between the first tangent to the peak and the baseline of the peak.

Table 1 tabulates the onset induction time measured for materials with various amounts of tristearin. Several trends can be noted in this data set. First and most obvious is the reduction in the induction time (indicative of a higher nucleation rate) with increasing undercooling, i.e., nucleation at lower isothermal temperatures. Only one set of three experimental replicates \((n = 3)\) is shown in Table 1. The results were not averaged due to the variability between each set of experimental replicates. However, the trends within each set of experimental replicates are similar. Each set of replicates was used to derive nucleation parameters. These parameters are averaged and reported as such.

Second, for the same nucleation temperature, the data shows that the addition of increasing amounts of tristearin reduces the induction time for nucleation. To illustrate this, contrast Figures 5 and 6. This effect is particularly pronounced for the high temperature (28 °C) although at the lowest temperature (23 °C), a reduction in the induction time can still be observed. This trend supports the hypothesis that the addition of SSS to the SOS preparation results in fractional crystallization of SSS prior to the crystallization of SOS. The availability of SSS surfaces and the affinity of SOS for this surface results in heterogeneous nucleation, which is manifested as an increase in the nucleation rate (reduction in the induction time), relative to a case where SSS is absent.

It must be noted that the reduction in the induction time appears to exhibit a minimum at a concentration of 3% SSS across all temperatures. This trend must be interpreted with...
caution. The differences are minimal and may likely not be significant in a statistical sense, particularly since the measurement of the induction time can be variable between different DSC pans of the same material. Indeed, repeated measurements of the induction time of new preparations of the material can show large variability (~60 s).

The data for Shea Stearin 1 + 1% SSS at 28 °C shows that at sufficiently high temperatures and low amounts of SSS, the induction time is either comparable to that of the material with no added SSS. An explanation for this phenomenon in Shea Stearin 1 may be that, at such low concentrations of SSS, and at such a high nucleation temperature, SSS is not super-saturated (if at all) to the extent that it crystallizes fractionally before SOS nucleates. This is evident via the absence of a small crystallization peak in the isothermal measurement step (Figure 7) for Shea Stearin 1 + 1% SSS at 28 °C. As such, even though tristearin is compositionally present in the material, no surfaces are formed that would promote heterogeneous nucleation and thus no reduction in the induction time is observed. This observation further supports the case for heterogeneous nucleation since the absence of surfaces (crystallization of SSS) does not lead to a reduction in the induction time.

**Fisher–Turnbull Results.** Table 2 shows the slopes m determined via linear regression of $\ln(\tau_{nucleation})$ vs $1/\left(T_{nucleation} \cdot (\Delta T)^2\right)$ as per the Fisher–Turnbull model. A melting point $T_f$ for SOS of 34.76 ± 0.13 °C ($n = 4$) and a melting enthalpy of 99.99 ± 2.49 J/g ($n = 4$) was used. This melting point was determined from the peak temperature of melting traces of the nucleated SOS (Figure 8), which were typically between 35 and 36 °C. This temperature is roughly the melting point of the $\beta'$-3 polymorph (form IV) of SOS, which was determined to be 36.5 °C. This suggests that the SOS crystallizes into the $\beta'$-3 polymorph directly from the melt. Over time (>2 weeks), the SOS undergoes a polymorphic transformation into the $\beta_1$-3 polymorph (form VI), which is manifested as a higher peak melting temperature (Figure 9). The melting point of the $\beta_1$-3 polymorph was determined to be approximately 43.0 °C.

The Fisher–Turnbull data show that as the amount of SSS is increased, the slope m decreases. The slope m can be interpreted as the constant of proportionality of the inverse relationship between $\Delta G_c$ and $(\Delta T)^2$. That is, in the case of a lower slope, the concomitant change in the activation free energy $\Delta G_c$ is smaller for each unit of change in the supercooling $\Delta T$. This suggests that as the amount of SSS is increased, the change in $\Delta G_c$ becomes less supercooling-dependent. The relationship between $\Delta G_c$ and $\Delta T$ is not linear but rather parabolic due to the squaring of the supercooling term.

The calculated interfacial free energy values $\gamma_{s-l}$ are relatively low (by approximately 1 order of magnitude) when compared to the $\gamma_{s-l}$ values determined by Phipps using the Fisher–Turnbull equation. The values determined by Phipps were on the order of ~10 mJ/m². However, values determined by Ahmadi and others using the same methodology ranged from approximately 0.1 to 5.0 mJ/m².

**Figure 6.** Nucleation peaks and determined induction times for Shea Stearin 1 + 3% SSS.

**Figure 7.** Absence of the fractional SSS peak in Shea Stearin 1 + 1% SSS nucleated at 28 °C.
The data in Table 2 suggests that there are three regimes (as separated into three sections in the table) of nucleation behavior. In the first region corresponding to 0% SSS, the slope attains a relatively high value, which indicates that the nucleation rate in the absence of SSS is very temperature-dependent. This consequently results in relatively high activation free energies at the given crystallization temperature. In the second region encompassing SSS concentrations between 1 and 2%, the slope decreases. This suggests that the addition of SSS reduces the supercooling dependence of the nucleation event. Consequently, the activation free energies and interfacial free energies at a given supercooling are lower than that of the material with no SSS added. In the region corresponding to SSS concentrations between 3 and 4%, the slope further decreases, which results in a reduction in the activation free energy of nucleation.

This is reflected in the induction time data in Table 1, which shows that the induction times for the concentrations between 3 and 4% are essentially identical. These trends are likewise reflected in the interfacial free energies, which decrease as the amount of SSS is increased. Since the calculated interfacial free energies can be assumed to be a weighted sum of the interfacial free energies for homogeneous and heterogeneous nucleation, a decrease in the interfacial energy with increasing SSS contents indicates a greater preponderance of heterogeneous nucleation (increased weight to heterogeneous nucleation). The data can therefore be interpreted in the following manner. At concentrations of 1–2% SSS, the amount of SSS surface formed is relatively low compared to the material with 3–4% SSS. As such, the amount of available surface for heterogeneous nucleation is much lower in the material with 1–2% SSS than the material with 3–4% SSS. The amount of material that can be heterogeneously nucleated in the material with 1–2% SSS is thus lower and this is reflected in the lower interfacial free energies.

Estimation of the Critical Radius. Table 3 tabulates the heterogeneous critical radius (assuming a cylindrical geometry) of an SOS island nucleating on an SSS surface (for materials with 1–4% SSS), assuming an effective $\gamma_{li}$ provided in Table 2. The critical radius decreases with decreasing nucleation temperatures (higher supersaturation) and increasing SSS contents. The first result is expected from nucleation theory: as
the supersaturation increases, the critical radius decreases. This results in a higher nucleation rate since the size of stable nuclei that grow on to become crystals is now smaller. The second result explains the increase in the nucleation rate upon the addition of SSS. In the presence of SSS surfaces, the surface free energy penalty is lowered via a reduction of the crystal-melt area due to the SSS surface. This results in an overall reduction in the activation free energy as well as a decrease in the size of the critical nuclei, both of which contribute to an increase in the nucleation rate.

Assuming that the lateral unit cell parameters of a similar triglyceride in the \( \beta' \) polymorph (2-oleodilaun, LaOLA) can be applied to SOS, the number of molecules in a cylindrical island of SOS can be estimated. The unit cell of the \( \beta' \)-3 polymorph of 2-oleodilaun (comprised of two LaOLA molecules) has lateral dimensions of 5.450 Å \times 7.736 Å with a cross-sectional area of 42.16 Å\(^2\). The smallest critical nuclei (Shea Stearin 1 + 3% SSS at 23 °C) has a cross-sectional area of 48.74 Å\(^2\). This area corresponds approximately to the cross-sectional area of a single unit cell. Thus, a critical nucleus in this scenario would contain approximately 2–4 SOS molecules. At the other extreme, the largest critical nuclei (Shea Stearin 1 at 28 °C) would have a cross-sectional area of 296.05 Å\(^2\), which is equivalent to roughly five unit cells. This would correspond to approximately 10–12 SOS molecules.

**MATERIALS AND METHODS**

**Atomic-Scale Molecular Dynamics.** The simulations conducted in this work are largely based on the methods of MacDougall and others, who utilized atomic-scale molecular dynamics (ASMD) to examine the ability of certain elaidic triglycerides to bind to SSS and trilaurin (LLL) surfaces. This study was conducted with a view to examining the oil binding ability of certain triglycerides on planar triglyceride surfaces. The force field used in the cited work was developed by Berger and others for modeling phospholipid membranes.

In the present work, the binding of stearic acid triglycerides (SOS, OOS, OSS, and SOO) to a planar crystalline TAG surface was studied with a view to elucidating how oil binding to an SSS surface can possibly lead to heterogeneous nucleation. The free and open-source molecule modeling package GROMACS 4.0 was used to conduct ASMD. The simulation workflow consisted of three major steps:

1. Energy minimization
2. Compression
3. Equilibration

A GROMACS trajectory file (*.gro) was first constructed by constructing an arbitrarily large simulation box and then populating it with the particles/molecules to be studied. This involved populating 40 molecules of the TAG to be studied in a milieu of triolein (OOO) particles at a ratio of 1:4 TAG/OOO. The trajectory file contains information on the positions and particle velocities of the molecules being studied. The molecules are separated from other molecules by relatively large distances to avoid overlap that may lead to spuriously high energies. Since the bonding information is not stored in the .gro file, two atoms may be introduced in a manner such that the position of the atoms correspond to a high energy state—a stretched double bond or two atoms overlapping in their repulsive shells. Such spuriously high energy states may introduce artefacts. Energy minimization is conducted on a trajectory file to impose the structural and energetic constraints (the topology) found in the .top file on the simulated particles. An energy minimization algorithm is then run to ensure that the entire system achieves a minimum free energy state prior to the actual “production run” simulation. A compression run is then conducted to fix the parameters of the system prior to the production run as well as to initialize the velocities of the molecules being simulated. In this case, this involved compressing the system to a fixed volume and temperature. In a compression run, the dimensions of the simulation box are slowly “compressed” to the final simulation box parameters. This is done slowly to avoid spuriously high energies.

The final equilibration run is the true production run that simulates the phenomenon being studied. This run generates the trajectory files used in the final analysis. ASMD works on the basis of numerically integrating Newton’s laws of motion over a large number of very “fine” time steps. In this work, the time step used was 1 fs \((10^{-15} \text{ s})\). The simulation was run for 10 000 000 steps, corresponding to a total simulation time of 10 ns. The simulation temperature was 52 °C or 325 K. At this temperature, the studied triglycerides (in the physical world) are in a molten state. A leapfrog integrator was used. A “heat bath” in the form of velocity rescaling was used to control temperature drift due to the imprecision introduced by dropping digits.

The simulation was carried out under the canonical NVT ensemble using GROMACS. The total energy \( U \) was calculated for every 500 frames of the simulation and reported to an .edr file. The Helmholtz free energy (applicable to an NVT ensemble) was calculated using the GROMACS utility g_energy_d. The calculated free energies were averaged over the last 5, 4, 3, 2, and 1 ns of the simulation to ascertain the trend of the free energies. By the time the simulation is half-completed, the free energies would have settled to an equilibrium value. GROMACS calculates the Helmholtz free energy \( \Delta A \) against an ideal gas initial state:

\[
\Delta A = A(N, V, T) - A(N, V, T)_{\text{gas}} = kT \ln \frac{U_{\text{pot}}}{kT}
\]

where \( k \) is Boltzmann’s constant, \( T \) is the system temperature and \( U_{\text{pot}} \) is the potential energy term of the total internal energy. The averaged free energy was divided by the total number of molecules \((N = 200)\) to calculate the free energy per molecule.

To determine the binding of the studied triglycerides to an SSS surface, the free energy per molecule of systems with different distributions of the studied triglyceride was studied. The triglyceride was modeled in two types of configurations: layered and mixed. In the layered configuration, the particles corresponding to the triglycerides were all placed close to the SSS surface as a molecular layer during the construction of the .gro file. This corresponds to a case where the triglycerides are bound to the SSS surface. In the mixed case, the triglycerides are dispersed throughout the simulation box, which represents a case where the triglycerides are not bound to the SSS surface. The free energy per molecule of the layered and mixed distributions was calculated.

The free energy per molecule of a reference state consisting only of 200 triolein molecules was subtracted from the free energies of the layered and mixed distributions. The difference in the free energy per molecule between the studied state and the triolein reference state was approximately 1–2 kJ/mol. The root-mean-square fluctuations around the average were plotted as the error of the averages. In cases where the error bars...
overlap, the chemical potentials of the two distributions are not considered to be different with the conclusion being that there are no free energy differences between the layered and mixed states. Where the error bars do not overlap, the two states are considered to have a free energy difference, which can be interpreted as a preference for one configuration (i.e., layered or mixed) relative to the other.

**Nucleation Kinetics. Experimental Materials.** Experimental work was conducted to test the hypothesis generated by the simulations that the presence of an SSS surface in a supersaturated melt will accelerate the nucleation of SOS via heterogeneous nucleation.

The work utilizes a purified shea stearin material dubbed Shea Stearin 1. Shea Stearin 1 was obtained from unpurified shea stearin supplied by the Fuji Oil Company (Savannah, GA). Purification was affected via a three-stage solvent fractionation process. Acetone was used as the fractionating solvent. At each step, the feedstock shea stearin was dissolved in acetone at mass ratios of 1:4. The dissolved fat was held at the fractionation temperature overnight to crystallize the SOS. Fractionation was initially conducted at a temperature of 16 °C to concentrate the SOS in the crystalline phase. The crystallized mass was then filtered and dried. Subsequent purification of this material was conducted by repeating this process at a temperature of 22 °C and then 25 °C. The latter two steps removed lower-melting TAGs, which further increased the concentration of SOS in the material.

The triglyceride composition (Table 4), in % w/w, of Shea Stearin 1 was characterized using isocratic reversed-phase high-performance liquid chromatography (Alliance Model 2690 Separation Module, Waters Corporation, MA) utilizing a C18 column (XBridge C18, 5 μm pore size, 4.6 × 250 mm2, Waters Corporation, MA) and a refractive index detector (Waters model 2410 RID, Waters Corporation, MA). The fractionated shea stearin was dissolved in 0.6 mL of chloroform and 1 mL of the mobile phase (60:40 acetone/acetonitrile). The tristearin (SSS, >99% purity) utilized in this work was sourced from Sigma-Aldrich.

**Table 4. Triglyceride Composition of Shea Stearin Materials Used in This Study**

| material         | POP | SOO | POS | SOS | SSS | unidentified |
|------------------|-----|-----|-----|-----|-----|--------------|
| Shea Stearin 1   | 1.5 | 6.1 | 4.8 | 79.8| 0.20| 7.6          |

**Measurement of Nucleation Rates Using Differential Scanning Calorimetry.**

The steady-state nucleation rate \( I_{SS} \) of a crystallizing fat system can be estimated experimentally by measuring the induction time for the appearance of the first crystalline material in a fat melt. The nucleation rate \( I_{SS} \) can be formulated as the inverse of the induction time \( \tau \):

\[
I_{SS} \sim \frac{1}{\tau}
\]

Traditionally, the induction time is measured using a phase transition analyzer (PTA), which utilizes a laser turbidometer to detect the appearance of crystalline nuclei invisible to the naked eye. The induction time is taken as the time at which the baseline signal increases by an arbitrary percentage (usually 5–10%). However, this method is not well-suited for the study of fractional crystallization in fats, as is the case of SSS in cocoa butter or SOS. This limitation is due to the inability of the PTA to resolve the separate fractional crystallization events. As determined using the PTA, the induction time will invariably be attributed to the crystallization of the first component, which will be the SSS component in the mixture, whereas interest in this study is in measuring the nucleation rate of SOS, which crystallizes as the second later fraction.

To distinguish between these separate crystallization events, differential scanning calorimetry (DSC) was used. If the SSS is present at a high enough concentration (≥1% SSS), the two triglyceride species can usually be distinguished as two distinct peaks in the crystallization exotherm with a smaller SSS peak crystallizing before SOS does, whereas in the absence of SSS, only one peak is observed.

The induction times of the various fats are measured using the following time–temperature program:

1. Heating at 30 °C/min to 80 °C.
2. Isothermal hold at 80 °C for 5 min to ensure complete melting.
3. Cooling to a temperature of crystallization using variable cooling rates to ensure that this cooling step is consistently 2 min for each of the various crystallization temperatures studied.
4. Isothermal hold at the crystallization temperature.
5. Heating at 5 °C/min to 80 °C to obtain the melting trace.

**Fisher–Turnbull Determination of the Activation Free Energy of Nucleation.** The Fisher–Turnbull model allows for the calculation of the activation free energy of nucleation \( \Delta G_\alpha \), given estimates of the nucleation rate (as the inverse of the induction time) at several different supersaturation/supercooling \( \Delta T \) (\( T_\text{i} - T \)). The Fisher–Turnbull equation is an expression for the steady-state nucleation rate \( I_{SS} \):

\[
I_{SS} = N \left( \frac{\gamma}{9 \pi} \right)^{1/2} \frac{J_0 k_B T}{h} \frac{1}{n_{0e}^{2/3}} \left( \frac{\Delta G_i}{k_B T} \right)^{4/3} \left( \frac{\Delta G_s}{k_B T} \right)^{-4/3}
\]

where \( N \) is the total number of molecules of the un-nucleated parent phase, \( A \) is the interfacial free energy per unit area and is calculated as the interfacial free energy divided by the surface area of the nucleus, \( n_{0e} \) is the number of particles on the surface of the critical nucleus, \( \Delta G_i \) is the activation Gibbs free energy associated with the diffusion of a nucleating particle across the phase boundary and its deposition onto the nucleating phase and \( \Delta G_s \) is the Gibbs free energy of nucleation. The term \( k_B T/h \) is the vibration frequency of the un-nucleated parent phase.

At temperatures corresponding to low supersaturation (or low undercooling), the \( -\Delta G_4 \) term is essentially constant and can be expressed as the pre-exponential \( \alpha \):

\[
I_{SS} = \alpha N \left( \frac{A}{9 \pi} \right)^{1/2} \frac{J_0 k_B T}{h} n_{0e}^{2/3} \left( \frac{\Delta G_i}{k_B T} \right)^{4/3} \left( \frac{\Delta G_s}{k_B T} \right)^{-4/3}
\]

At low supersaturation (or low undercooling), the dominant term that describes the rate of nucleation is therefore the \( \Delta G_4 \) term. However, at high supersaturation, the \( \Delta G_4 \) term (diffusion being a thermally activated process) becomes significant and exerts a considerable influence on the nucleation rate.

A plot of \(-\ln \frac{I_{SS}}{\tau} \) vs \( \frac{1}{(\Delta T)^4} \) will yield a straight line with a slope equivalent to:
where $V_M$ is the molar volume of the solid phase (m$^3$/mol), $\gamma_{c-1}$ is the solid–liquid interfacial tension (J/m$^2$), $T_f$ is the melting point or temperature of fusion (K), $\Delta H_m$ is the molar enthalpy of fusion (J/mol), $\Delta G_c$ is the activation free energy of nucleation (J), and $\Delta T$ is the undercooling (K), equivalent to the difference between the melting point $T_f$ and the experimental temperature of nucleation $T_i$, $T_f - T_i$.

Alternatively, a plot of $\ln T_i' vs. (1/(\Delta T))$, where $T_i'$ is the induction time in seconds, will also yield the same results. From the slope of the Fisher–Turnbull plot, the activation free energy of nucleation $\Delta G_c$ at various supercoolings can be calculated as follows:

$$\Delta G_c = \frac{mk_b}{(\Delta T)^2}$$

(9)

With good calorimetric data of the melting point $T_f$ and the melting enthalpy $\Delta H_m$ as well as estimates of the molar volume $V_M$, the liquid–solid interfacial free energy per unit area $\gamma_{c-1}$ can be calculated according to the following calculation:

$$\gamma_{c-1} = \left(\frac{3mk_b(\Delta H_m)^3}{16\pi(V_M^2(T_f)^2)}\right)^{1/3}$$

(10)

Performing this calculation for materials containing SSS would allow for the calculation of an "effective system-wide" interfacial free energy per unit area which would exhibit a reduction if heterogeneous nucleation were to occur. The effective interfacial free energy per unit area is assumed to be a weighted average of the interfacial free energy of homogeneous nucleation and heterogeneous nucleation. As such, the extent of the decrease in this effective interfacial energy is reflective of the prevalence of heterogeneous nucleation.

Estimation of the Critical Radius. With an estimate of the interfacial energy, the size of the critical nuclei for heterogeneous nucleation can be calculated according to the method presented by Ramel and others. This model assumes that TAG molecules nucleate on a surface as a cylindrical "island" (Figure 1). The height of this cylinder is equivalent to the lamellar height of the nucleating species, which is the length of the molecular axis of the particular polymorph the nucleating species crystallizes into. Using the model provided, an expression for the critical radius of this cylindrical island can be derived:

$$r_c = \frac{\gamma_{c-1}(T_f)(V_M)h}{(\Delta H_m)(\Delta T)h - \gamma_{c-1}(T_f)(V_M)h}$$

(11)

For the homogeneous nucleation of a cylinder in the absence of a surface, the expression for the critical radius is:

$$r_c = \frac{\gamma_{c-1}(T_f)(V_M)h}{(\Delta H_m)(\Delta T)h - 2(\gamma_{c-1})(T_f)(V_M)}$$

(12)

For this work, the values used are given in Table 5.

The melting point $T_i$ and melting enthalpy $\Delta H_m$ were obtained from the DSC melting trace (scanned at 5 K/min) of the Shea Stearin 1 crystallized into the $\beta'$ polymorph. The molar volume $V_M$ was calculated from density data of the $\beta'$ polymorph of SOS provided in the work by Arishima and others.

The lamellar height $h$ was calculated from the (003) reflections of the powder X-ray spectra of Shea Stearin 1 crystallized into the $\beta'$ polymorph. The X-ray spectra were obtained using a MultiFlex Powder XRD spectrometer (Rigaku, Tokyo, Japan) with a copper X-ray source (wavelength of 1.54 Å) operated at 40 kV and 44 mA. The measurement scan rate was set at 0.1°/min between the range $2\theta = 1–30^\circ$ at 20 °C. Peak positions were determined using MDI Jade 9 (MDI, Livermore, CA) software. The obtained value was found to be in agreement with literature values.

**Table 5. Parameters Used in the Calculation of Nucleation Characteristics**

| parameter   | value |
|-------------|-------|
| $T_i$       | 34.76±0.13°C or 307.91±0.13 K (n = 4) |
| $\Delta H_m$| 99.99±2.49 J/g or 88.939±2.214 kJ/mol (n = 4) |
| $V_M$       | 9.02 m$^3$/mol |
| $h$         | 70 Å |

**CONCLUSIONS**

The hypothesis formulated using computer simulations was demonstrated via nucleation kinetics studies of SOS and SSS mixtures. Differential scanning calorimetry showed that the addition of SSS to SOS preparations resulted in an increased nucleation rate of the bulk of the SOS. Higher amounts of added SSS led to greater reductions in the induction time, which can be interpreted as an increase in the amount of surface on which heterogeneous nucleation may occur. Estimation of the surface free energy showed that the surface free energy was reduced via the addition of SSS, which supports the hypothesis of the heterogeneous nucleation of SOS. The reduction of the surface free energy in heterogeneous nucleation also led to a decrease in the activation free energy and consequently, the critical nuclei radius. These would contribute to the increase in the nucleation rate.

The heterogeneous nucleation of SOS in the presence of SSS has significant implications for the processing and crystallization of fats in confectionery products. For example, it is commonly known that the activation free energy of the $\alpha$ and $\beta'$ polymorphs is lower than that of the desired $\beta$ (form V) polymorph. The presence of SSS and its promotion of heterogeneous nucleation, which implies a lowered activation free energy, may have an unintended effect on the nucleation of undesirable $\alpha$ and $\beta'$ polymorphs during cocoa butter tempering. A lowered activation free energy implies that a higher rate of nucleation can be sustained with lower supersaturation. Bearing this in mind, the presence of SSS crystals may promote the re-nucleation of these undesirable polymorphs as the tempered cocoa butter mass is inevitably cooled in the final stages of processing. Furthermore, the high melting point of SOS will lend persistence to these effects since the SSS crystals are not melted during the melting phase of the tempering procedure. The impact of SSS crystals on the processing of chocolate is worth further investigation.

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Notes
The authors declare no competing financial interest.

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