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Efecto del Superenfriamiento y la Viscosidad en la Formación de Redes Cristalinas en Sistemas Modelo a Base de Triacilglicéridos Puros y Aceites Vegetales.

Trabajo recepcional en su opción
Artículos de Investigación

Que como parte de los requisitos para obtener el grado de

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Presenta

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EFECTO DEL SUPERENFRIAMIENTO Y LA VISCOSIDAD EN LA FORMACIÓN DE REDES CRISTALINAS EN SISTEMAS MODELO A BASE DE TRIACILGLICÉRIDOS PUROS Y ACEITES VEGETALES

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RESUMEN

Se estudió el comportamiento de mezclas de tripalmitina (TP) y triestearina (TS) 25:75 con trioleína (TO), aceites de cártamo alto en oleico (HOSfO) y de soya (SBO) previo a la nucleación de triacilgliceroles (TAGS) empleando espectroscopia de fluorescencia polarizada (FPS) y simulación mecánica molecular (MM). Adicionalmente, se determinó la energía libre de activación para la nucleación (ΔG_c) utilizando la ecuación de Fisher-Turnbull. Las temperaturas de cristalización empleadas (ej., 36°C-41°C en mezclas-TP y 46°C-51°C en mezclas-TS) se seleccionaron para obtener condiciones de superenfriamiento similares en ambas mezclas. Los resultados fueron estadísticamente analizados por ANOVA contrastando las medias de los tratamientos. La anisotropía de las mezclas de TP:HOSfO y TP:SBO aumentó a diferencia de la TP:TO y todas las mezclas -TS. Este comportamiento se asoció a un aumento en la microviscosidad de la fase líquida como consecuencia de la estructuración de los TAGS previo a su nucleación. Se realizó MM para entender las interacciones moleculares responsables de este comportamiento lo que indicó que las fuerzas de van der Waals son las responsables del aumento en la microviscosidad. Existe una interacción específica favorable particularmente cuando el aceite contiene TAGS con al menos un ácido palmítico, lo que induce en las mezclas una estructuración de los TAGS antes de la nucleación. En las mezclas-TS esto ocurrió antes de alcanzar las condiciones de superenfriamiento requeridas para la cristalización. Por consiguiente, la ΔG_c en las mezclas TS:HOSfO y TS:SBO fue menor que la ΔG_c de la TS:TO. En contraste, en las mezclas-TP, la estructuración de los TAGS ocurrió en función del tiempo bajo condiciones isotérmicas. Postulamos que las interacciones moleculares que ocurren en la fase líquida entre la TP y los TAGS con ácido palmítico resultan en el desarrollo de estructuras lamelares líquidas de TAGS mixtos, resultando en una mayor ΔG_c a medida que la concentración de TAGS con ácido palmítico aumenta. Es necesario entender las interacciones moleculares entre TAGS en la fase líquida ya que están relacionadas con la cristalización qué, a su vez, determina las propiedades macroscópicas de productos lipídicos. Este trabajo versa en esta línea de investigación.

Palabras clave: Nucleación de triacilgliceroles, cristalización, espectroscopia de fluorescencia polarizada, mecánica molecular, energía libre de activación.

SUMMARY

We have studied the pre-nucleation behavior of tripalmitin (TP) and tristearin (TS) blended in a 25:75 ratio with triolein (TO), high oleic safflower oil (HOSfO) and soybean oil (SBO) using fluorescence polarization spectroscopy (FPS), and molecular mechanics simulations (MM). We also determined the activation free energy to develop a stable nucleus (ΔG_c) using the Fisher-Turnbull equation. The crystallization temperatures used (i.e., 36°C-41°C in TP blends, and 46°C-51°C in TS blends) were selected to obtain similar supercooling conditions in both types of blends. The results were statistically analyzed using ANOVA and contrast among the treatment means. The FPS measurements showed that there is an increase in the anisotropy of TP:HOSfO and TP:SBO blends as opposed to TP:TO and all TS blends. This behavior is directly associated with an increase in the microviscosity of the liquid phase as a result of triacylglycerols (TAGS) structuring prior to the crystals' nucleation. We performed MM to understand the molecular interactions responsible for this behaviour. The results indicated that short range van der Waals interactions are responsible for the increase in microviscosity prior to TAGS crystallization. There seems to be a specific favourable interaction particularly when the oil contains TAGS with at least one palmitic acid in their composition, inducing a pre-nucleation structuring in the blends. In TS blends the molecular interaction occurred well before attaining supercooling conditions required for crystallization. As a result, ΔG_c in TS:HOSfO and TS:SBO blends were lower than the ΔG_c in the TS:TO blend. In contrast, in the TP blends TAGS structuring occurred as a function of time under isothermal conditions. We postulate that the molecular interactions occurring in the liquid phase between TP and TAGS with palmitic acid resulted in the development of mixed TAGS lamellar liquid structures. This resulted in higher ΔG_c for TP nucleation as the concentration of TAGS containing palmitic acid increased in the blends. We need to understand the molecular interactions among TAGS in the liquid phase, since they relate to the TAGS crystallization process that in turn determines the macroscopic properties of lipid-based products. This study is included in this line of research.

Keywords: Triacylglycerols nucleation, crystallization, fluoresence polarization spectroscopy, molecular mechanics, activation free energy.

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Í N D I C E

Página

I. INTRODUCCIÓN

INTRODUCCIÓN

Los aceites y grasas vegetales son sistemas multicomponentes que contienen distintas familias de triacilglicéridos (TAGS). En estos sistemas las relaciones moleculares que se producen entre las familias de TAGS determinan las condiciones termodinámicas (ej., superenfriamiento y supersaturación), que impulsan la formación de un sólido de la fase líquida, la organización de la fase sólida y su comportamiento de fase. La red tridimensional de cristales de TAGS resultante y su fase de comportamiento son factores importantes para determinar las propiedades físicas y funcionales (ej., reología, entrampamiento de la fase líquida, palatabilidad, apariencia y untabilidad) en productos tales como margarina, mantequilla y recubrimientos o rellenos para pasteles. Así, las complejas interacciones moleculares que se producen entre TAGS en la fase líquida bajo condiciones de superenfriamiento deben ser primero entendidas, ya que se relacionan directamente con el proceso de cristalización de TAGS que, en tiempo, determina las propiedades macroscópicas evaluadas por el consumidor en aceites vegetales y productos a base de grasa.

En principio todos los líquidos son isótropos: los movimientos moleculares dentro del líquido son brownianos por naturaleza y pueden ser descritos con la relación de Stokes–Einstein. Sin embargo, bajo ciertas circunstancias el fluido puede mostrar un movimiento preferencial o tener una restricción en su orientación. Es entonces dicho que el líquido está estructurado y por lo tanto muestra anisotropía. Ejemplos de estos líquidos estructurados son los cristales líquidos (LC). Su estado, ya sea proviniendo de la solución (liotrópico) o del fundido (termotrópico), es entre los límites de líquidos isótropos y cristales sólidos. A este estado también se le conoce como mesofase o bien estructura mesomórfica. Las propiedades ópticas anisotrópicas de los

cristales líquidos de fluidos poliméricos son como las de los sólidos regulares, pero sus moléculas presentan movimientos libres similares a las de los líquidos. Una de las particularidades de estas estructuras es que muestran una anisotropía espontánea y son fácilmente orientadas, siempre y cuando se encuentren como LC. Esta es una propiedad muy útil ya que un alto grado de alineación molecular en el estado líquido puede determinar las propiedades del producto final solidificado. Se ha sugerido que los TAGS también pueden mostrar anisotropía cuando se encuentran en estado de LC. El comportamiento de la fase de TAGS ha sido objeto de varios estudios debido a la importancia del polimorfismo en el procesamiento técnico de las grasas. Al respecto, diversos investigadores han propuesto las fases nemática, sméctica y discótica como disposiciones de arreglo molecular estructural para TAGS en fase líquida. Aunque no han establecido la organización estructural mesofásica de los TAGS y han utilizado como modelos sistemas simples, estas investigaciones apoyan el hecho de que las moléculas de TAGS de alguna manera están estructuradas en el estado líquido cristalino.

Debido a la aplicabilidad de los TAGS en mezclas multicomponentes en la industria alimentaria, cosmética y farmacéutica, la presente investigación es un intento para estudiar la estructura en estado líquido antes de la nucleación de sistemas más complejos. Los sistemas empleados fueron diseñados para proporcionar diferentes grados de compatibilidad molecular entre diferentes fases de disolventes (ej., trioleína, aceite de cártamo alto en trioleína y aceite de soya) con los TAGS saturados de interés (ej., tripalmitina y triestearina). Además, el tamaño y la forma de las estructuras de los LC cambian con la velocidad de difusión de las moléculas. Esto se traduce en un cambio en la viscosidad del sistema como función de la temperatura. Así, la viscosidad del aceite es una medida indirecta de la estructura o la

anisotropía de los TAGS en estado líquido, de manera que, cuando los aceites vegetales son enfriados, la viscosidad aumenta debido a una mayor organización de los TAGS. En estudios previos se ha demostrado que hay un aumento en la anisotropía (ej., medida por fluorescencia polarizada) en función del enfriamiento de los aceites, este aumento está relacionado al incremento en la viscosidad del medio. Por lo tanto, las medidas combinadas de viscosidad y anisotropía en aceites vegetales proporcionan información relacionada a la estructura líquida previa a la nucleación y su relación con la cristalización. Paralelamente al estudio de las interacciones moleculares que ocurren en los TAGS antes de su nucleación, investigamos su posible involucramiento en la energía libre de activación ΔG_c) requerida para desarrollar un núcleo estable.

Por otro lado, las interacciones responsables del aumento en la anisotropía de un fluido son moleculares por naturaleza. El conocimiento de estas interacciones puede ser explorado por medio de simulaciones. En este trabajo, se realizó una simulación mecánica molecular (MM) para modelar el sistema con el fin de entender el papel de la energía involucrada en alcanzar un estado particular. MM es un método sencillo que describe la energía implicada en un sistema por medio de un conjunto de derivadas clásicas de funciones potenciales. En particular, a través de simulaciones, evaluamos aquí la estructura líquida de un sistema modelo binario de TAGS antes de la cristalización. Por lo tanto, creemos que el uso de simulaciones MM para estudiar mezclas complejas de TAGS (ej., grasas) cristalizados, proporcionaría información acerca de las interacciones energéticas responsables de la estructuración en estado líquido justo antes de que la nucleación se lleve a cabo.

II. RESUMEN EN EXTENSO Y PRIMER ARTÍCULO

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ARTÍCULO ORIGINAL

Estructuración Previa a la Nucleación de TAG Fundidos por Medio de Espectroscopia de Fluorescencia Polarizada y Simulación Mecánica Molecular

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RESUMEN

En la presente investigación fue estudiado el comportamiento previo a la nucleación de la tripalmitina (TP) y la triestearina (TS) en mezclas 25:75 con trioleína (TO), aceite de cártamo alto en oleico (HOSfO) y aceite de soya (SBO), por medio de Espectroscopia de Fluorescencia Polarizada (FPS) y simulación Mecánica Molecular (MM). La composición de los aceites fue determinada por cromatografía líquida de alta resolución (HPLC) y reportada para los principales triacilglicéridos (TAGS), como la media (± desviación estándar) de al menos tres determinaciones independientes. Las temperaturas de cristalización empleadas (ej., 36°C a 41°C en mezclas de TP y 46°C a 51°C en mezclas de TS, con aumentos de grado en grado) fueron determinadas por calorimetría diferencial de barrido (DSC) y seleccionadas para obtener condiciones de supersaturación similares en ambas mezclas. Así mismo, de los termogramas correspondientes fueron obtenidas las propiedades térmicas de las mezclas. El estado polimórfico de las muestras cristalizadas fue obtenido por difracción de

rayos-X aplicando barridos de 1°/min desde 1° a 40° 2θ. La viscosidad de las muestras durante la etapa de enfriamiento previa a la cristalización isotérmica fue obtenida cada 5°C con un espectrómetro mecánico equipado con sistema True-Gap, mientras que la anisotropía de las mezclas fue calculada usando las intensidades de fluorescencia de las mezclas previamente marcadas con 1,6-difenil-1,3,5-hexatrieno (DPH). Las intensidades fueron medidas en un espectrofotómetro de fluorescencia polarizada equipado con un monocromador de alta velocidad y cámara Peltier para control de temperatura. Los resultados fueron estadísticamente analizados por ANOVA contrastando las medias de los tratamientos. Por último, para obtener mayor información del grado de estructuración previo a la nucleación fueron realizadas simulaciones mecánicas moleculares empleando el software Chem3D. En todas las simulaciones fueron usadas 16 moléculas agrupadas en 4 filas de 4 moléculas, como se muestra en la figura 1.

Los termogramas con las propiedades térmicas de las mezclas mostraron dos endotermas, la primera a menor temperatura, asociada a la fusión de los TAGS de la TO, HOSfO y SBO, y la segunda a la mayor temperatura, correspondió a la fusión de la TP o TS según el sistema. La Tabla 1 resume las temperaturas de fusión de la segunda endoterma y sus correspondientes entalpías. Es claro que el TAG saturado en cada caso, funde a la misma temperatura para las tres mezclas correspondientes: 58 °C y 65 °C para las mezclas TP y TS respectivamente, siendo esto un indicador de que no hay interacciones específicas entre la TP o TS y los solventes utilizados.

Todos los patrones de difracción obtenidos mostraron picos aproximadamente a 19°, 23° y 24° correspondiendo estos a los espacios de 4.60, 3.85 y 3.70 Å, indicando que las mezclas cristalizaron como polimorfos β (ej., estructura triclínica). Como ejemplo, la figura 2

muestra difractogramas a temperaturas seleccionadas para mezclas de TP (Fig. 2a) y TS (Fig. 2b).

Las viscosidades obtenidas durante la fase de enfriamiento fueron graficadas en función de la anisotropía para cada una de las mezclas. La tabla 2 muestra los valores de la pendiente, la ordenada al origen y el coeficiente de correlación de las linealidades obtenidas. Los altos valores de R^2 muestran que la viscosidad y la anisotropía varían linealmente, de aquí que la anisotropía, en el rango estudiado, es una medida de la viscosidad del volumen del sistema.

Aplicando la ecuación de Perrin modificada por Weber y col. para moléculas no esféricas, fueron calculadas las viscosidades de las mezclas para verificar la validez de esta aproximación. La tabla 3 muestra los valores de la viscosidad calculada y la determinada experimentalmente, ambas están en el mismo orden de magnitud, no obstante, la microviscosidad calculada es de manera consistente aproximadamente la mitad de la viscosidad experimental. La causa podría ser que, físicamente, la microviscosidad es un concepto diferente a la viscosidad del volumen de la mezcla, especialmente si el fluido está, de alguna manera, estructurado. Sin embargo, el resultado también podría implicar que la ecuación de Perrin no ajusta al sistema.

Los resultados de FPS a diferentes temperaturas mostraron que la anisotropía de las mezclas de TP:HOSFO y TP:SBO aumentó a diferencia de la TP:TO (Fig. 3). Este comportamiento fue directamente asociado a un aumento en la microviscosidad de la fase líquida como efecto de interacciones moleculares que conducen a la estructuración de la mezcla previa a la nucleación y crecimiento de los cristales. El efecto del solvente en las muestras de TP es diferente en cada caso. El que la mezcla de TP:TO no muestre incrementos implica que la estructuración del fluido no es debida a la presencia de trioleína en la mezcla. Según nuestra propuesta, esto debe ser un efecto debido a la presencia de

otros TAGS presentes en el HOSFO y el SBO. Además, los picos de anisotropía son mayores para las mezclas TP:SBO, indicando que el efecto de ordenamiento fue más fuerte en presencia de SBO como solvente en las mezclas. Para este efecto fue determinada la composición de TAGS en las mezclas (Tabla 4). En el caso de las mezclas TS, algunas temperaturas mostraron aumentos en la anisotropía pero los más consistentes fueron en mezclas con SBO. Podríamos hipotetizar que, de alguna manera, la presencia de uno o diferentes TAGS presentes en SBO inducen el efecto de ordenamiento en el fluido y, de estar presente, la concentración no fue lo suficientemente alta para lograr una estructuración en las mezclas TS:HOSfO.

Se realizó MM para entender las interacciones moleculares responsables del comportamiento de la anisotropía en las mezclas. El componente van der Waals (vdW) para un sistema de TP pura una vez minimizada la energía, fue de 695 ± 4 kcal/mol. En contraste, la simulación de la TO pura resultó tener un valor de 747 ± 2 kcal/mol. Esto indica que las fuerzas vdW son las responsables del comportamiento líquido o sólido de los TAGS y por lo tanto, causantes del aumento en la microviscosidad previo a su cristalización. La figura 4 muestra el promedio de los valores de la energía vdW para diversas mezclas de TP y sus análogos de TS. Las mezclas TP:PLL, TP:PLO y TP:POO tuvieron una energía vdW significativamente menor ($P > 0.05$) que la mezcla TP:TO. En las mezclas de TS se encontró un comportamiento similar. Estos resultados indican también, que la presencia de moléculas que contienen al menos una cadena de ácido palmítico en su composición de TAGS, induce a un aumento en la microviscosidad de la mezclas previo a la nucleación. Por último, se estudió la aplicabilidad de estas conclusiones a TAGS de cadena larga. Nuestros resultados de MM mostraron que las mezclas hipotéticas de TS

y TAGS conteniendo ácido esteárico en su estructura, no tendrá una energía vdW lo suficientemente baja como para dar cuenta de un aumento en la microviscosidad.

En conclusión, este trabajo estudió el ordenamiento previo a la nucleación que ocurre en mezclas de TAGS saturados con diferentes aceites líquidos. Los experimentos de FPS nos dieron una visión sobre la estructuración a nivel microscópico que ocurre en esta etapa. Sin embargo, no estaba claro cuales interacciones eran las responsables de este efecto a nivel molecular. Se realizaron simulaciones de MM para abordar este aspecto. MM es una herramienta rápida y simple, pero poderosa, que puede proporcionar información complementaria a los datos experimentales. Obviamente, los resultados obtenidos necesitan ser cuidadosamente interpretados según las condiciones usadas en las simulaciones. Una vez que esto se logra, el resultado de aplicar este procedimiento es generalmente una mejor comprensión de las interacciones moleculares responsables de un determinado fenómeno observado experimentalmente. Utilizando MM hemos sido capaces de determinar que las interacciones de van der Waals son claves en la etapa anterior a la nucleación de grasas. También hemos demostrado que ocurre un comportamiento específico en función de la composición molecular de un TAG en particular. Estos resultados indican que las interacciones tanto generales como específicas desempeñan un papel importante en la etapa previa a la nucleación.

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ORIGINAL PAPER

Pre-Nucleation Structuring of TAG Melts Revealed by Fluorescence Polarization Spectroscopy and Molecular Mechanics Simulations

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Abstract We have studied the pre-nucleation behavior of tripalmitin (TP) and tristearin (TS) in blends with triolein (TO), high oleic safflower oil (HOSfO) and soybean oil (SBO) by means of fluorescence polarization spectroscopy (FPS) and molecular mechanics simulations (MM). The FPS measurements at different temperatures showed that there is an increase in the anisotropy of the TP:HOSfO and TP:SBO blends as opposed to the TP:TO sample. This increase is directly related to an increase in the microviscosity of the blend which is interpreted as a structuring step prior to the nucleation and growth of the crystals. A similar but less pronounced effect was also observed in the TS:SBO blends. We performed MM simulations in an attempt to understand the molecular interactions responsible for this behavior. The simulation results have shown that short range van der Waals interactions are the ones responsible for the increase in the microviscosity of the blends prior to crystallization. Our results also indicate that the presence of molecules that contain at least one chain of palmitic acid in their triglyceride (TAG) composition will induce a pre-nucleation increase in the microviscosity of the blend in both TP and TS containing systems. Lastly, we studied the applicability of these conclusions to longer chain TAG analogues. Our MM results show that hypothetical blends of TS and TAGs containing stearic acid in their structure, will not have a low enough vdW energy to account for an increase in the microviscosity. Hence, there seems to be a specific interaction particularly favorable when the oil contains TAGs with at least one palmitic acid chain.

Keywords Triglycerides · Structuring · Pre-nucleation · Fluorescence polarization spectroscopy · Molecular mechanics

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Introduction

In principle all liquids are isotropic: the molecular motions within the fluid are Brownian in nature and can be described by the Stokes–Einstein relationship. However, under certain circumstances a fluid may display a preferential motion or have an orientational restriction. It is then said that the fluid is structured and hence displays anisotropy [1]. Examples of these structured liquids are liquid crystalline (LC) polymers. The state of their solution (lyotropic) or melt (thermotropic) is between the boundaries of solid crystals and isotropic liquids. This state is also referred to as a mesomorphic structure or a mesophase. The anisotropic optical properties of liquid crystal polymeric fluids are like those of regular solids, but their molecules are free to move similar to liquids. One of the many interesting properties of these mesogenic structures is that they show spontaneous anisotropy and are easily oriented, provided that they are in a LC state. This is an extremely useful property because a high degree of molecular alignment in the fluid state can determine the properties of the solidified end product. This is essential,

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for example, for making high stiffness materials such as Kevlar (DuPont), Vectra (Hoechst Celanese) and Xydar (Amoco) among other classical examples.

It has been suggested that triacylglycerides (TAGs) also display anisotropy when in the LC state. The phase behavior of TAGs has been the subject of several studies due to the importance of polymorphism in technical fat processing.

Several groups have proposed different molecular arrangements for TAG melts. Namely, nematic and smectic phases have been the two mesophases most argued over on this subject. Cebula et al. [2] have proposed that TAGs in melts arrange themselves in a nematic phase characterized by molecular symmetry axes lined up parallel but without any positional order. Larsson, on the other hand, has postulated that the lipid molecules in the LC state arrange themselves in a lamellar-type of order typical of a smectic phase, where the molecules posses some positional order [3]. Sato and co-workers [4, 5] have shown through synchrotron radiation X-ray diffraction that TAGs in which one of the chains is unsaturated, display a LC phase characterized by lamellar ordering. However, their results are for pure TAGs that were quenched to temperatures well below the corresponding melting point and then heated rapidly to the target temperature. More recently, Corkery et al. [6] have proposed that molten TAGs form a discotic phase in which the triglyceride molecules exist in the liquid state with fully splayed chains, approximating "Y''-shapes. Regardless of the actual mesophase, all the work mentioned above agrees on the fact that, TAG molecules are somehow structured in a LC-like state [2–9]. However, they all deal with model, single TAG component, samples. Due to the applicability of multicomponent TAG blends in food, cosmetic and pharmaceutical industries, the present work is an attempt to study the structure in the liquid state, prior to nucleation of these more complex systems. Our systems are meant to provide different degrees of molecular compatibility between different solvent phases [triolein, safflower oil high in triolein, and soybean oil (SBO)] with the saturated TAGs of interest (tripalmitin and tristearin).

The shape and size of these LC structures changes with the diffusion rate of the molecules [8]. This translates into a change in the viscosity of the system as a function of temperature. Thus, oil viscosity is an indirect measurement of the structure or anisotropy of TAGs in the liquid state. When vegetable oils are cooled, the viscosity increases due to a higher organization of the TAGs [10]. Marangoni has shown that an increase in the anisotropy, as measured by fluorescence polarization, is observed upon cooling of oils, which is correlated to an increased viscosity of the medium [11]. Hence, the combined measurements of viscosity and anisotropy of vegetable oils provide information regarding the liquid structure in a pre-nucleation state and its relationship with crystallization.

Fluorescence polarization is a technique used to study the rotational movement of molecules in suspensions as well as in solutions [12] and provides information about concentration, binding events, and molecular structure, diffusion, mobility on membranes, and structure on surfaces [13, 14]. In fluorescence polarization spectroscopy (FPS), a fluorophore is used as a probe to study the hydrodynamic behavior at the molecular level. Briefly, as the stiff fluorophore molecules in a fluid diffuse throughout the bulk, they undergo a precessional motion characterized by a rotational correlation time, Φ. This is a time constant that characterizes the molecular rate of rotation. When polarized light impinges an ensemble of fluorophores, only those that are aligned with the plane of polarization will be excited. The emission can then undergo two different paths depending on the lifetime of the process: If the fluorescence lifetime of the excited fluorescent probe is much longer than the rotational correlation time of the molecule it is bound to, the molecules will randomize in solution during the process of emission. As a result, the emitted light of the fluorescent probe will be depolarized. If, on the contrary, the fluorescence lifetime of the fluorophore is much shorter than the rotational correlation time, the excited molecules will stay aligned during the process of emission. In this case the emission will be polarized. Hence, the extent of depolarization is a measure the rotational correlation time. Because the molecules experience intermolecular friction forces that oppose their rotational diffusion, the extent of the depolarization is a measure of the anisotropy of the system and, in turn, of its microviscosity. A great deal of theoretical and empirical expressions have been derived for this purpose and the reader is referred to the works of Marangoni [11], Royer [15] and Mann and Krull [16] for a comprehensive review of the technique.

The interactions responsible for an increase in the anisotropy of a fluid are molecular in nature. The knowledge of these interactions can be explored by means of computer simulations. In this work, we have used molecular mechanics (MM) to model the system in order to understand the role of energetics in achieving a particular state. MM is a simple method which describes the energy of the system in terms of a set of classically derived potential functions.

In particular, we evaluate here the liquid structure of binary model systems of TAGs prior to crystallization through MM simulations. Similar studies have been carried out in the past in different systems. For example, Nagarajan and Mayerson [17] have used MM to understand the role of energetics in the formation of the helical conformation of isotactic polypropylene in an attempt to understand

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nucleation in the early stages of crystallization. Interestingly, the work of Sato and co-workers [5] on monounsaturated TAGs notes that the ordering sequences between the lamellar stacking and the lateral packing of these molecules is very similar to that found upon crystallization of isotactic polypropylene [18]. Hence, we believe that using MM simulations to study complex TAG blends (e.g. fats) crystallized under non-isothermal conditions, would provide information regarding the energetic interactions responsible for the structuring in the liquid state just before nucleation takes place.

Materials and Methods

Triacylglycerides, Vegetable Oils, and Blend Preparation

Tripalmitin (TP), tristearin (TS), and triolein (TO) with purities higher than 99% were obtained from Sigma Chemical Co. (St. Louis, MO). Refined, bleached and deodorized high oleic safflower (HOSfO) and SBO were obtained from local manufacturers. TAG composition of the oils was determined by HPLC following the procedure described by Perez-Martinez et al. [19] The composition of major TAGS was reported as the mean (±standard deviation) of at least three independent measurements. Each of the saturated TP and TS were blended with either TO, HOSfO, or SBO in a 25:75 (wt:wt) ratio. After 20 min at 80 °C with constant gentle stirring, the blends were stored under nitrogen atmosphere at 2 °C in amber glass vials.

DSC Measurements

The crystallization and melting thermograms of the blends were obtained by DSC using a TA Instruments Model Q2000 (TA Instruments, New Castle, DE, USA). After calibration of the equipment, blend samples (6–8 mg) were sealed in aluminum pans, heated at 80 °C for 20 min and then cooled to −60 °C at a rate of 10 °C/min. After 2 min at this temperature the samples were heated to 80 °C at a rate of 5 °C/min. In all cases an empty pan was used as reference. The melting temperature (T_m) and enthalpies were calculated from the melting thermograms using the equipment software.

X-Ray Diffraction

X-ray diffraction patterns of the crystallized blends (14 mg) were obtained using a Rigaku Multiplex Powder X-ray diffractometer (Rigaku, Japan) using a Cu source X-ray tube ($\lambda = 0.1542$ nm) at 40 kV and 44 mA. After crystallizing the blend at the corresponding temperature using a cooling rate of 10 °C/min, the X-ray diffraction was recorded at scanning rate of 1° per min from 1° to 30° 2θ. The temperature was controlled with a Peltier system and the results were analyzed using MDI's Jade 6.5 software (Rigaku, Japan).

Viscosity Measurements

The viscosity of the blends investigated was obtained at different temperatures with a mechanical spectrometer (Physica MCR 301, Anton Paar, Stuttgart, Germany) equipped with TruGap system (P-PTD200/TG) using a 2.5-cm diameter parallel-plate geometry (PP25/TG). The temperature was controlled by a Peltier system located in both the base and top of the measurement geometry through a Peltier-controlled hood (H-PTD 200). The control of the equipment was made through the software Start Rheoplus/32 version 2.65 (Anton Paar, Graz-Austria). After 20 min at 80 °C the system was cooled at a rate of 10 °C/min. At each 5 °C interval during the cooling stage, the shear stress was determined by applying a logarithmic shear rate sweep from 1 to 100 per s. Before the measurements the system was allowed to achieve temperature equilibrium for 5 min. The viscosity (η) was determined as the slope of shear stress versus shear rate by linear regression. Two independent determinations were obtained for each temperature.

Anisotropy Measurements, During the Cooling Stage, Under Isothermal Crystallization Conditions

The anisotropy (r_s) of the blends was calculated using the fluorescence intensities of blends previously labeled with a 1 mM of 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma, St. Louis, MO, USA) solution in tetrahydrofuran (2 µl/ml). The intensities were measured with a polarizer spectrophotometer (MD-5020 of Photon Technology International, London, Ontario, Canada) equipped with a high speed random access monochromator DeltaRAM using the FeliX32 software. The prefixed wavelength was 360 nm for excitation and 465 nm for emission. The fluorescence intensities of the blends (250 µl samples) were obtained during the cooling (i.e. as a function of temperature) and the isothermal stages (i.e. as a function of time) with the observation polarizer parallel or perpendicular to the incident plane-polarized light. The two intensities were obtained in independent experiments under the same time–temperature conditions. In each case two independent determinations were carried out. For the TP blends, the anisotropy was measured every degree from 36 to 41 °C, and for the TS blends every degree from 46 to 51 °C. Although the range of temperatures is different for the TP and TS blends, the supersaturation conditions are very

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similar. The supersaturation ($\ln\beta$) of the melts was calculated as

$$\ln \beta = \frac{\Delta H}{RTT_m}\Delta T \quad (1)$$

where R is the gas constant, T the isothermal crystallization temperature. ΔH the enthalpy of fusion, T_m the melting temperature for either TP or TS, and ΔT is the difference between the melting temperature and the temperature of the experimental run [20]. For the anisotropy experiments, $\ln\beta$ ranges from 0.16 to 0.25 and from 0.15 to 0.24 for the TP and TS blends respectively. These ranges indicate that all samples had similar supersaturation conditions.

Temperature control was achieved with a Peltier system attached to a four position cuvette holder (Turret 400, Quantum Northwest). The anisotropy value (r_s) was calculated under the cooling and isothermal stages of the blend crystallization considering the G correction for light scattering as described by Lakowicz [21]. The G correction factors for the TP blends were 1.06, 1.02, and 0.97 for TO, HOSfO, and SBO, respectively; and 1.09, 1.03 and 0.96 for the TS blends with TO, HOSfO, and SBO, respectively.

Construction of the MM Initial Configuration

The general considerations to develop these TAGs lamellar structures used the following criteria:

- Saturated TAGs molecules had to be stackable in a chair-like conformation with parallel hydrocarbon chains at positions *sn*-1 and *sn*-3 and anti-parallel at *sn*-2 with a thickness of 2*L*. Due to the unsaturation present in oleic acid this was more complicated in the case of triolein (TO) than for the fully saturated TP, and even more so for TAGs having different acyl chains such as PLL or PLO.
- For unsaturated TAGs two possible conformations were explored, such that the hydrocarbon chains at positions *sn*-1 and *sn*-3 were pointing (a) in the same direction as the *sn*-2 chain, and (b) away from the hydrocarbon chain at *sn*-2 position.
- All unsaturations were in *cis* conformation
- Changes in the average tilt angle of the TAGs packing for the three major polymorphs, α, β' and β, had no effect on the energy minimization procedure.

Once these requirements were fulfilled, the actual initial configuration was found such that changes in dihedral angles of each TAG in a stack of four molecules led to a very similar minimization energy. This was taken as an indication of a minimum energy configuration. It should be noted that the vdW energy falls off as r^{-6}, but it can be cut off at much shorter distances. In this work, a cutoff value of 10 Å was used throughout, which increased the speed of the computation significantly.

All the simulations were performed with CS Chem3D Pro (CambridgeSoft v. 3.5.1), 16 molecules were used, stacked in 4 rows of 4 molecules, as shown in Fig. 1. The packing was such that the van der Waals radii of the atoms of adjacent molecules were just in contact with each other. The rows were arranged in both alternating and "sandwiched" conformation. In the latter, two rows of the same molecule were in between two rows of the other molecule. The final result of the energy minimization for these two types of conformation was statistically determined to be equivalent. Hence we used these to calculate an average of 4–6 sets for different mixes of TAGs.

Mixtures of more than two types of TAG proved to be extremely difficult to simulate as the number of variables in the initial configuration increased the uncertainty of the minimization procedure.

Once the initial configuration was set, the MM2 (an all atom force field based on the MM1 functional form [22]) energy minimization was allowed to run until a root mean squared (RMS) gradient value of 0.014 was attained using a steepest descent minimization algorithm. If the slope of the potential energy surface becomes small enough, then the minimization has probably reached a local minimum on the potential energy surface, and the minimization terminates. Our convergence criterion was a good compromise between accuracy and speed of the simulation.

Results

Differential Scanning Calorimetry and X-ray Analysis

DSC measurements were performed on all blends. The melting thermograms (data not shown) consistently showed two endotherms. The lower temperature endotherm is associated with the melting of the TAGs from TO, HOSfO and SBO. The higher temperature endotherm corresponds to the melting of either TP or TS depending on the system. Table 1 summarizes the temperature values for the second endotherm and the corresponding enthalpies of melting as determined from the integration of the peak. It is clear that the fully saturated TAG in each case melts at about the same temperature for the three corresponding blends: about 58 and 65 °C for the TP and TS blends respectively. This is an indication that there are no specific chain–chain interactions between the TP, or TS, and the solvents used in each mixture affecting the solubility and phase behavior of the blends.

The crystallinity of the blends was assessed by X-ray diffraction. Figure 2 shows the X-ray spectra for selected temperatures for both the TP blends (Fig. 2a) and the TS

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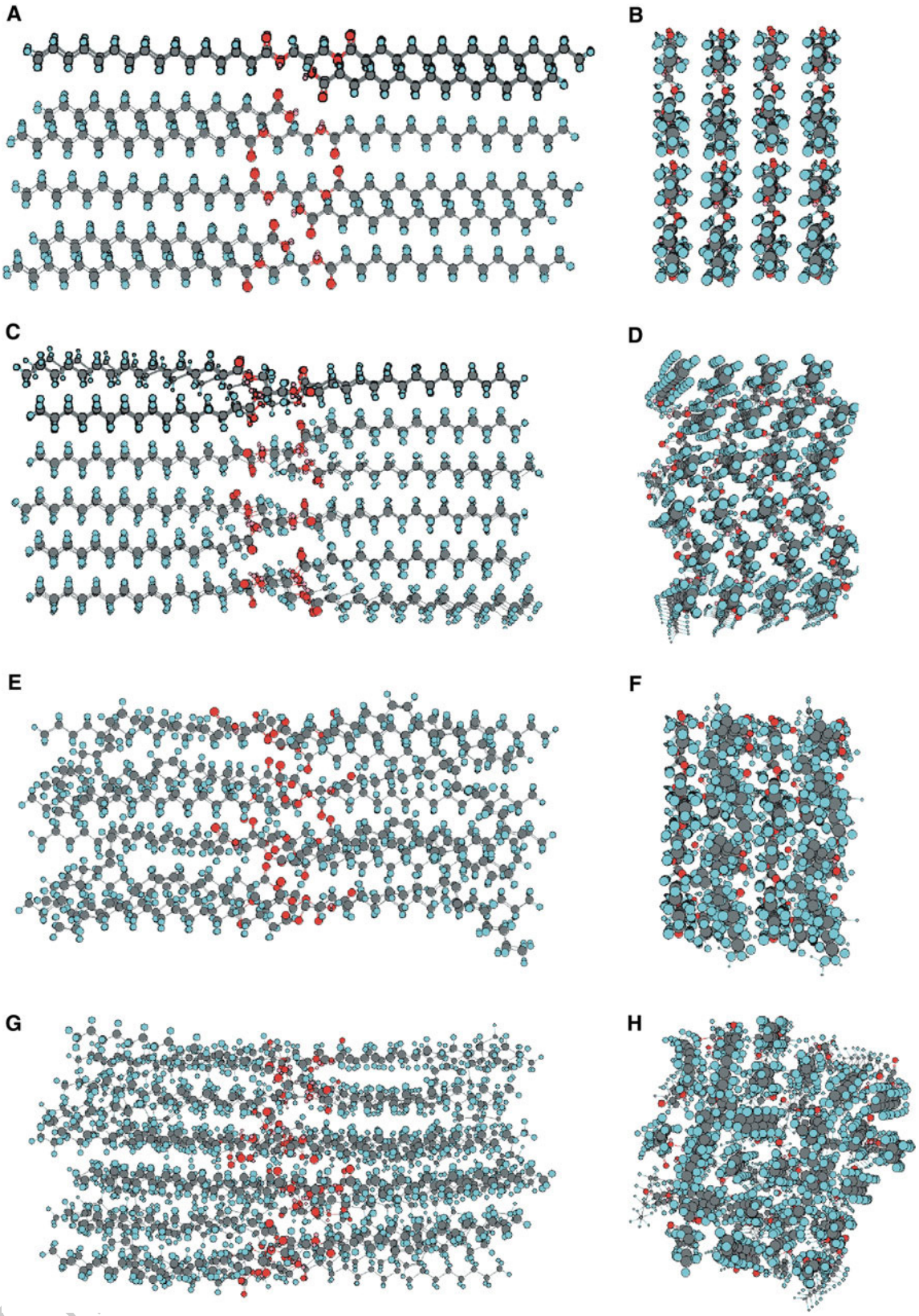

Fig. 1 Stack of 16 molecules arranged in 4 rows of 4 molecules each. **a**–**d** For a TP stack. **e**–**h** For a TP:PLO mix in a 1:1 molecular ratio. The initial configurations are seen from a side (**a**, **e**) and front (**b**, **f**) perspectives. After the energy minimization side (**c**, **g**) and front (**d**, **h**) views are shown for comparison

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Table 1 Melting temperatures and enthalpies of fusion for the TP and TS in their respective blends as determined from DSC heating measurements

Blend	T_m (°C)	ΔH (kJ/mol)
TP:TO	57.6 ± 0.4	10.4 ± 0.3
TP:HOSfO	57.5 ± 0.4	10.58 ± 0.2
TP:SBO	57.60 ± 0.01	11.39 ± 0.01
TS:TO	65.17 ± 0.09	11.89 ± 0.01
TS:HOSfO	64.7 ± 0.5	12.8 ± 0.1
TS:SBO	65.0 ± 0.1	12.93 ± 0.01

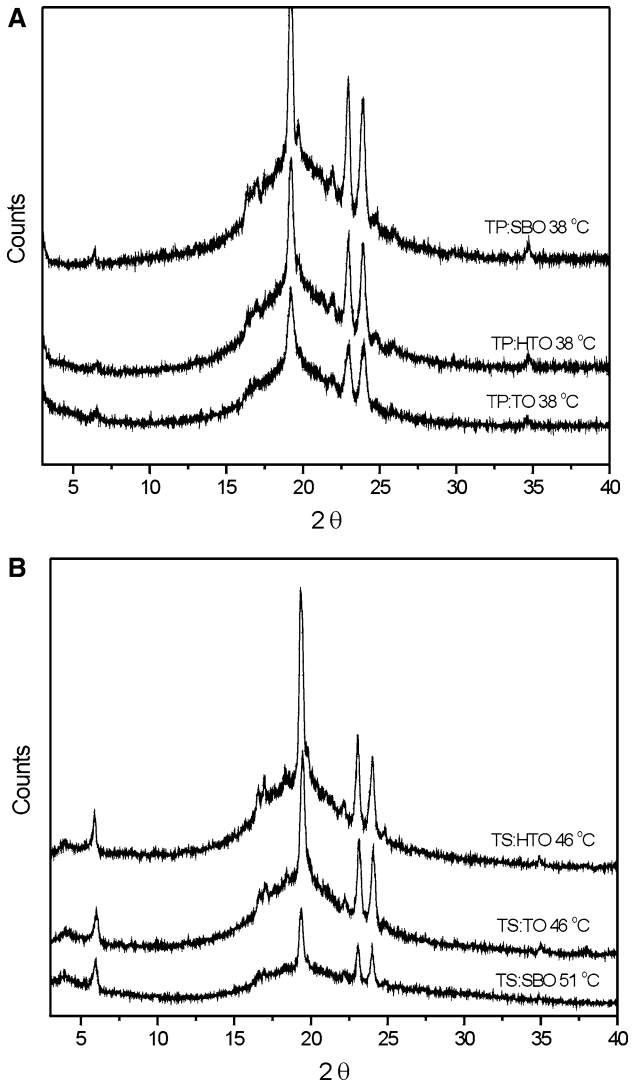

Fig. 2 X-Ray diffraction patterns. All TP blends (**a**) were crystallized at 38 °C. TS blends (**b**) crystallized at 46 or 51 °C as shown in the figure

blends (Fig. 2b). It is clear from the peaks at approximately 19°, 23° and 24° corresponding to spacings of 4.60, 3.85 and 3.70 Å, that the blends crystallized as the β polymorph (triclinic subcell structure).

Fluorescence Polarization Spectroscopy

We have plotted the viscosity of the system determined from rheological measurements as a function of the anisotropy for each of the blends. Table 2 shows the values of slope, intercept and correlation of the linearity for these plots. The high R^2 values clearly indicate that the viscosity and the anisotropy vary linearly, hence showing that the anisotropy, within the range studied, is a measure of the bulk viscosity of the system.

The Perrin equation has been modified by Weber et al. [23] to account for molecules that are not spherical (as is the case for most fluorescent tags), such that:

$$\frac{r_0}{r_s} = 1 + \frac{kT\tau}{\bar{\eta}v_0} \quad (2)$$

where r_s is the molecular anisotropy with r_o/r_s defined as the degree of depolarization, T the absolute temperature, τ the average lifetime of the excited state, k the Boltzmann constant, $\bar{\eta}$ is the microviscosity of the medium, and v_0 the effective volume of the fluorescent probe. The maximum anisotropy value r_o is close to 0.4 for DPH [24]. The van der Waals volume of the DPH probe has been reported to be 233 Å^3 [24]. We have used Eq. 2 to determine the viscosity of the blends and verify the validity of this approach.

Table 3 shows the values of viscosity calculated using the Perrin equation and those determined experimentally for all blends at 55 °C. The fluorescence lifetime, τ, of

Table 2 Slope, intercept and correlation coefficient for plots of viscosity versus anisotropy for the TP (45–80 °C) and TS (55–80 °C) blends

Blend	Slope	y-Intercept ($\times 10^{-3}$)	R^2
TP:TO	0.762	9.4	0.996
TP:HOSfO	0.807	8.3	0.982
TP:SBO	0.674	9.9	0.984
TS:TO	0.719	9.9	0.983
TS:HOSfO	0.852	10.3	0.938
TS:SBO	0.852	9.6	0.972

Table 3 Comparison between the bulk and calculated viscosities using Eq. 2 at 55 °C

Blend	Bulk viscosity η (Pa s)	Microviscosity $\bar{\eta}$ (Pa s)
TP:TO	0.0297	0.0129
TP:HOSfO	0.0301	0.0130
TP:SBO	0.0278	0.0129
TS:TO	0.0310	0.0139
TS:HOSfO	0.0303	0.0119
TS:SBO	0.0294	0.0110

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DPH is both temperature and solvent dependent. At 55 °C τ is approximately 10 ns in heavy white paraffin oil [11]. The calculated and experimental viscosities are in the same order of magnitude. However, there is a significant discrepancy between the two. The calculated microviscosity is consistently lower than the experimental values by approximately a factor of 2. The reason could be a physical one, in the sense that the microviscosity could be different than the bulk viscosity of a blend, especially if the fluid is somehow structured. However, this result could also imply that the Perrin equation used here does not really hold for our systems, due to some specific behavior of the blends. Regardless, it is interesting that both sets of values are in the same order of magnitude.

Anisotropy as a Function of Time

Measurements of the normalized anisotropy, $r_s/r_{t=0}$, at different supersaturation conditions are shown in Fig. 3a, b and c for the TP:TO, TP:HOSfO and TP:SBO blends, respectively and in Fig. 3d, e, and g for the TS analogues. All the plots display an initial period of time where the anisotropy is constant. Also, a decrease in the anisotropy at the end of the experiment is common to all measurements due to the scattering of light from newly formed nuclei. In some cases, the anisotropy increases during the course of the experiment before dropping at the end. Within these features, the behavior of the TP and TS blends is different. The time scale of the experiments for the TS samples is on average three times longer (about 20 min) than for the TP blends (between 5 and 7 min). This is an indication of a higher ΔG of nucleation for the TS blends, relative to the TP blends.

The effect of the solvent in TP samples is different in each case. TP:TO blends do not display an increase in the anisotropy at any of the temperatures studied. For the TP:HOSfO and TP:SBO samples, the measurements done at 37, 38 and 40 °C in the former blends and those at all temperatures for the latter one, display a clear increase in the anisotropy. This increase in the anisotropy is related to an increase in the microviscosity of the system and hence is an indication of molecular interactions leading to the structuring of the blend in a pre-nucleation state. The fact that the TP:TO mixes show no increase, clearly implies that the structuring of the fluid is not due to the presence of triolein in the blend. We propose that it must be an effect due to the presence of other TAGs present in the HOSfO and SBO. Furthermore, the peak in anisotropy is highest for the TP:SBO blends, indicating that the strongest ordering effect is in the presence of SBO as the blend solvent.

Several smaller features can be appreciated in Fig. 3. In the measurements of TP:HOSfO at 36 and 38 °C and in those at 46 and 47 °C for the TS:HOSfO mixes, after the initial decrease in anisotropy due to the appearance of crystals, the fluorescence polarization signal becomes very weak and unstable and sometime spiking behavior is observed due to artifactual scattering effects. The TAG composition of HOSfO and SBO was determined in our laboratory and it is summarized in Table 4. TAGs present in a concentration of less than 5% in at least one of the oils have been omitted from the table for simplicity. Based on our FPS results, the interactions that lead to an increase in the anisotropy are probably related to the presence of TAGs other than TO (OOO in the table) in the blends.

In the case of the TS blends, the general trend is slightly different to that seen in the TP samples. Firstly, the TS:TO samples at 49 and 50 °C show an increase in the anisotropy that it is not seen for any of the TP:TO blends. Secondly, the only increase in anisotropy seen in the TS:HOSfO blends is a spike-like behavior seen at 47 °C. However, the TS:SBO blends display a similar anisotropy increase to the one observed in the TP:SBO mixes if only for the higher temperature experiments. We believe that the increases seen in the TS:TO and TS:HOSfO blends are due to artifacts and hence, are not representative of the behavior of the blend, especially since they occur in the middle of the temperature range. As a consequence, the only blends that display some pre-nucleation ordering for the TS fat are those dissolved in SBO. This is again an indication that there is a TAG-specific interaction at the molecular level responsible for the structuring of the blend prior to crystallization. We could hypothesize that somehow the presence of one or several TAGs present in SBO induces this ordering effect of the fluid and that, if at all present, the concentration is not sufficiently high in the TS:HOSfO blends to allow for the structuring effect. However our results so far, do not allow for a better insight on the forces and conditions necessary for this pre-nucleation increase in the anisotropy to occur. We have used MM simulations to address this aspect.

Molecular Mechanics Simulations

We have performed MM simulations in an attempt to understand the molecular interactions responsible for the experimental behavior seen in the anisotropy measurements of the blends. The energies given by the Chem3D program are divided into bonded and non-bonded interaction energies. The latter one is further divided into Dipole/dipole, long and short range van der Waals interactions. Of these terms, only the short range van der Waals component showed a consistent trend in the results within the reproducibility of the method. The total energy and all other components given as a result of the minimization showed

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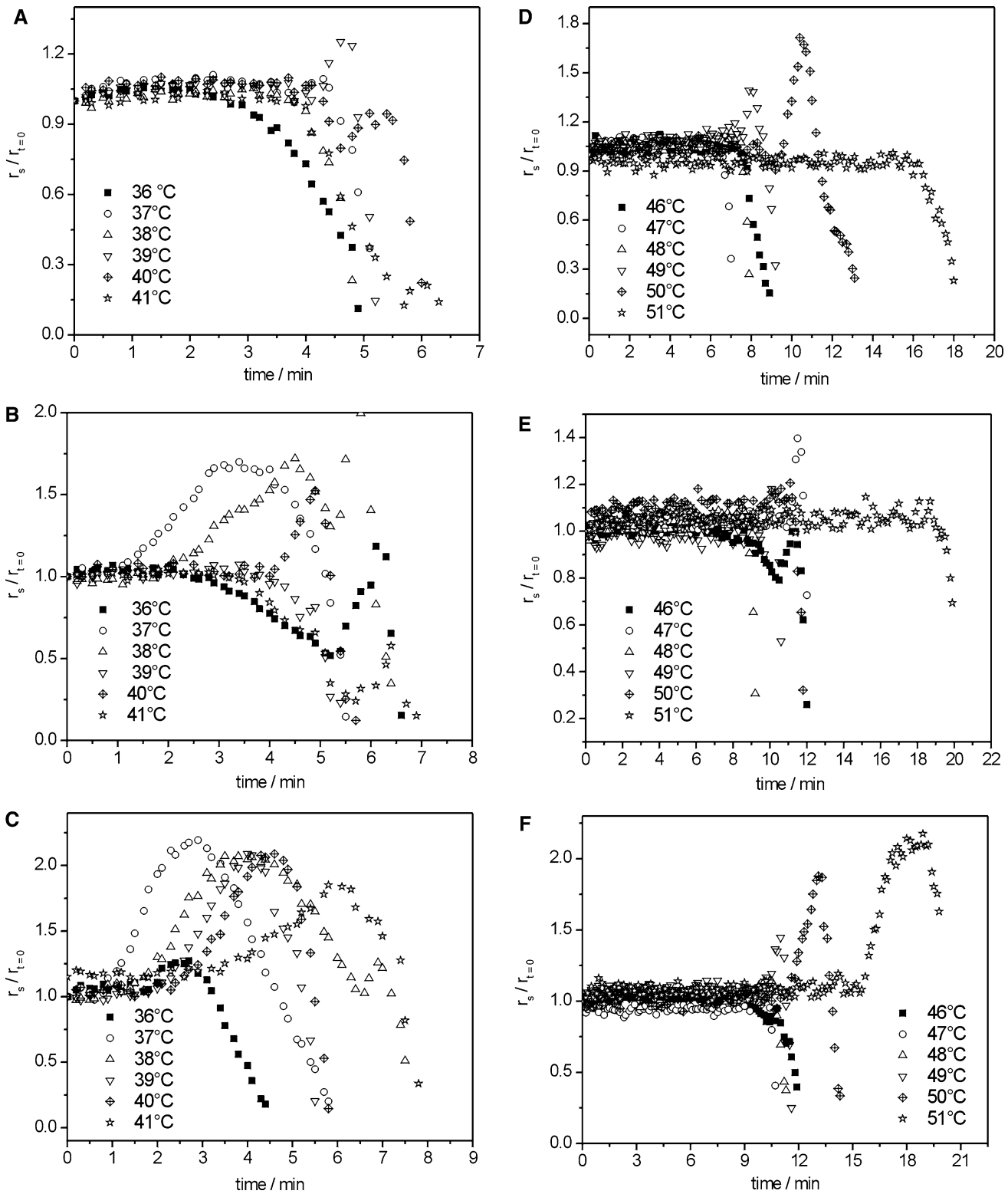

Fig. 3 Normalized anisotropy as a function of time for **a** TP:TO, **b** TP:HOSfO, **c** TP:SBO, **d** TS:TO, **e** TS:HOSfO and **f** TS:SBO at the temperatures indicated in each figure

no particular trend as a function of the type of molecule used. Hence, we refer hereon only to the short range vdW energy in order to explain our experimental results.

The vdW component of a pure TP system upon minimization was determined to be 695 ± 4 kcal/mol. In contrast, a simulation of pure TO resulted in a value of

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Table 4 TAG composition (%w/w) of safflower oil high in triolein (HOSfO) and soybean oil (SBO)

TAG	HOSfO	SBO
LLL	0.48 (0.05)	21.4 (0.5)
LLO OLL	2.24 (0.06)	21.2 (0.9)
LLP	0.26 (0.02)	14.5 (0.6)
LOO	16.20 (0.08)	9.9 (0.1)
PLO POL	1.6 (0.1)	10.42 (0.03)
OOO	65.4 (0.9)	3.67 (0.03)
POO	8.6 (0.3)	2.7 (0.3)

TAGs present in less than a 5% concentration in at least one of the oils have been omitted. Numbers in parentheses correspond to the standard deviation of four measurements

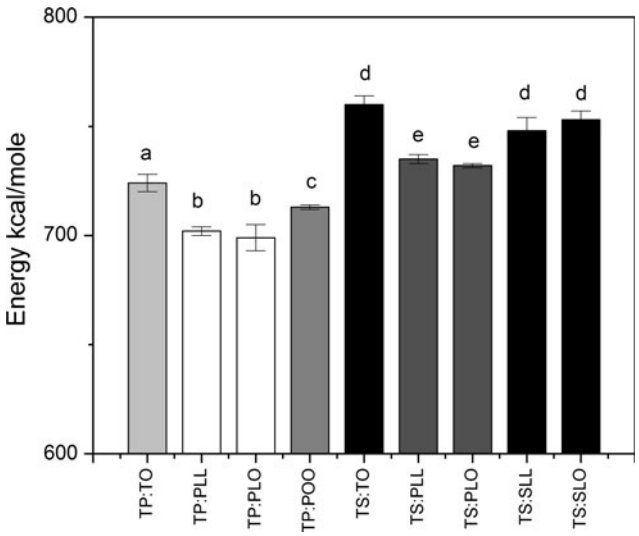

Fig. 4 Short range vdW interactions for different TAG mixes in a 1:1 M ratio. *Error bars* correspond to the standard deviation of 3–6 independent simulations with different starting configuration

747 ± 2 kcal/mol. This is an indication that vdW forces are somehow responsible for the liquid or solid behaviour of a TAG. Figure 4 shows the average vdW energy values for mixtures of TP:TO, TP:PLL, TP:PLO, TP:POO and the analogues in TS blends. PLL in our simulations is composed of one palmitic and two linoleic acid chains, PLO of one palmitic, one linoleic and one oleic acid chain and POO of one palmitic and two oleic acid chains. We have performed statistical analysis on our results to determine which mixes present a potential favorable vdW interaction. Also, different initial configurations of the same mix yield statistically equivalent results ($P > 0.05$). This then allowed for the calculation of an average value for different mixes and the determination of statistically significant differences between samples. The letters on the bars in Fig. 4 summarize our results. In this way, the TP:PLL, TP:PLO and TP:POO mixes have a significantly lower vdW energy than the TP:TO blend. A similar trend is seen for the TS:PLL and TS:PLO with respect to the TS:TO analogue. The same tendency was seen for mixtures in a 1:3 stoichiometry (data not shown). The last two bars correspond to blends of TS:SLL and TS:SLO. Although SLL and SLO exist in very small proportions in the oils, we have treated these blends with TS as "hypothetical". The purpose was to prove if the decrease in the vdW energy was related to the length of the TAGs involved or due to a specific effect related to the presence of palmitic acid in the TAG molecule. This last result seems to indicate that TAGs containing at least one saturated 16-carbon chain interact better with both TP and TS. Hence, the specificity does not arise from same length chains in the solute and solvent TAGs, but from a particular aspect of the palmitic acid.

Discussion

This work deals with the behavior of liquid oils in the presence of fully saturated TAGs. We have used both TP and TS with the purpose of comparing the effect that carbon chain length has on the behavior of the system. When a fluid has a preferential motion in a particular dimension or has an orientational restriction, it is said that the fluid is structured and hence displays anisotropy. This ordering of the fluid causes a change in the microviscosity of the system. Interestingly enough, this change in the microviscosity is highly correlated to the bulk viscosity of the system. Our fluorescence polarization results clearly indicate that there is a pre-nucleation structuring of the blend characterized by an increase of the anisotropy as a function of time as seen in Fig. 3. Our calorimetric data are supporting evidence that our FPS results are not due to a solubility or phase behavior effect, but rather a specific interaction of the blend components at the molecular level. In this work, we have ran single-component and bi-component MM simulations, to gain a better insight on the forces responsible for the pre-nucleation structuring seen in the FPS experiments.

The energies given by the Chem3D program are not related to any thermodynamic quantity, and have no meaning as absolute quantities. However, the values can be used for comparing relative behaviors between different conformations as done in this work. The total energy is divided into seven components: stretch, bend, stretch-bend combination, torsion, non-1,4 (or long range) van der Waals, 1,4 (or short range) van der Waals and dipole/dipole interactions. The sum of all these components corresponds to the total minimized energy of the system. Since bonded interactions are highly dependent on the initial configuration of the molecule, we have only used the terms

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corresponding to non bonded interactions in our initial assessment of the energy minimization. The non-bonded interactions given by the program correspond to long and short range van der Waals forces and dipole/dipole interactions. Of these, only the short range vdW interactions displayed a pattern as a function of the TAG molecule used in the mix. This is in agreement with the work of Sato et al. [4] who have suggested that van der Waals interactions are responsible for the lamellar structure seen in their XRD measurements on monounsaturated TAGs.

For either TP or TS, Fig. 4 clearly indicates that the vdW energies are highest for mixes with TO. Hence the interaction between these TAGs is the least favorable. We believe that this is the reason why our FPS experiments show no increase in the anisotropy for the TO blends. Simulations for mixes of PLL and PLO with both saturated fats, show a decrease in the vdW energy with respect to the TO samples. We have interpreted this as an indication of a particular behavior occurring in the presence of TAGs that contain at least one palmitic acid. This explains the experimental result obtained through FPS, in which mixtures of TP:HOSfO and TP:SBO showed an increase in the microviscosity. HOSfO has about 10% of TAGs containing palmitic acid in their composition whereas SBO has a content of close to 30% of these TAGs. We believe that it is the presence and abundance of these palmitic acid-containing TAGs what determines the increase in the microviscosity prior to the actual nucleation of fat crystals. Hence, although an increase in anisotropy is seen in the TP:HOSfO blends, the highest effect is seen for the TP:SBO and TS:SBO samples.

Our results suggest that blends of saturated fats in oils that contain at least 10% of TAGs with at least one chain of palmitic acid, will show an increase in the microviscosity as a pre-nucleation step in the crystallization process. One could now hypothesize that this result is extensible to longer chain analogues. Hence, to induce crystallization one needs a liquid oil that has a relatively high content of TAGs that have at least one saturated chain. Interestingly, the effect is very specific to palmitic acid. The MM simulations performed on hypothetical blends of TS:SLL and TS:SLO indicate that the even if the saturated chain length is the same as that of the solid fat, the interaction is not necessarily favorable enough to induce ordering in the pre-nucleation stage. Very recently, Vereecken et al. [25] have suggested on the basis of DSC measurements, that TP seems to play a more important role as seeding agent in the crystallization of POP than TS does for SOS. This interesting conclusion agrees with the specific behavior seen for the palmitic-TAGs with respect to the stearic-containing analogues.

Conclusions

This work has studied the pre-nucleation ordering occurring in blends of saturated TAGs in different liquid oils. Our FPS experiments have given us insight on the microscopic level structuring that occurs at this stage. However, it was not clear which interactions were responsible for this effect at the molecular level. We performed MM simulations to address this aspect. MM is a fast and simple, yet powerful, tool that can provide complementary information to experimental data. Obviously, the results obtained need to be carefully interpreted based on the conditions used for the simulations. Once this is achieved, the resulting outcome of this procedure is usually a better understanding of the molecular interactions responsible for a particular phenomenon observed experimentally. Using MM we have been able to determine that van der Waals interactions are key in the stage prior to the nucleation of fats. We have also shown that a specific behavior occurs depending on the molecular composition of a particular TAG. These results indicate that both general and specific interactions play a role in the pre-nucleation step. It is our hope that this work has set grounds for the implementation of MM simulations in similar systems to the ones here presented.

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III. RESUMEN EN EXTENSO Y SEGUNDO ARTÍCULO

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ARTÍCULO ORIGINAL

Estructuración Previa a la Nucleación de Triacilgliceroles y su Efecto Sobre la Energía de Activación para la Nucleación

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RESUMEN

Basados en un estudio previo que involucró mediciones de anisotropía (r_s) y simulación mecánica molecular (MM), en el presente trabajo fue investigada la interacción molecular producida entre los triacilgliceroles (TAGs) antes de su nucleación y su posible participación en la determinación de la energía libre de activación necesaria para desarrollar un núcleo estable (ΔG_c). La ecuación de Fisher-Turnbull fue utilizada para calcular la ΔG_c implicada en la nucleación de la tripalmitina (TP) o la triestearina (TS) mezcladas en una proporción 25:75 con trioleína (TO), aceite de cártamo alto en oleico (HOSfO) y aceite de soya (SBO). Las temperaturas de cristalización y fusión correspondientes así como los tiempos de inducción a la cristalización fueron determinados por calorimetría diferencial de barrido. Basados en los valores de la temperatura de fusión en el equilibrio ($T_M°$), las temperaturas de cristalización (T_{Cr}) utilizadas fueron seleccionadas para obtener condiciones de superenfriamiento similares en todas las mezclas de TP y TS investigadas. Así, el superenfriamiento fue calculado como $T_M°$-T_{Cr}. Para las mezclas de TP, el tiempo de inducción a la cristalización (t_i) fue medido desde 36 °C hasta 41 °C cada grado y, para las mezclas

de TS desde 46 °C hasta 51 °C cada grado. Los resultados fueron estadísticamente analizados por ANOVA (Statistica, V 9.0) contrastando las medias de los tratamientos. Por otro lado, la anisotropía de las mezclas fue determinada durante las etapas de enfriamiento e isotérmica, con un espectrofotómetro de fluorescencia polarizada empleando los mismos patrones tiempo-temperatura que para las mediciones de t_i. La r_s para las diferentes mezclas de TP y TS fue graficada en función de $T_M°$-T_{Cr}.

Las $T_M°$ para la TP y TS en sus tres mezclas fueron 59.7°C ($\pm$0.83) y 70.2°C ($\pm$0.27) respectivamente, ya que resultaron ser independientes del tipo de disolvente. Por otro lado, los correspondientes t_i y ΔG_c en función del superenfriamiento para las mezclas de TP y TS son mostrados respectivamente en las Tablas 1 y 2. A todas las T_{Cr} fue siempre obtenida una exoterma simple de cristalización que correspondió al polimorfo β para la TP o TS cristalizada en las mezclas. La regresión linear del $\log[(t_i)(T_{Cr})]$ vs. $1/T_{Cr}(T_M°\text{-}T_{Cr})^2$ dio en todos los casos coeficientes de determinación (R^2) mayores a 0.86 (ej., $R>0.927$). Como ejemplo, la figura 1 incluye las gráficas Fisher-Turnbull para las mezclas de TP (Fig. 1a) y de TS (Fig. 1b).

En general, para todas las muestras investigadas, el t_i y la ΔG_c incrementaron a medida que el superenfriamiento disminuyó. En todos los casos, para un superenfriamiento similar, t_i fue mayor en las mezclas de TS que en las mezclas de TP. Para las mezclas de TP a todos los niveles de superenfriamiento investigados, el t_i mayor fue para las mezclas TP/SBO (Tabla 1) mientras que la ΔG_c se vio incrementada siguiendo el orden SBO>HOSfO>TO (Tabla 2). Sin embargo, se hace notar que la ΔG_c para las mezclas de TP/SBO y TP/HOSfO no fue estadísticamente diferente a ninguna de las T_{Cr} investigadas ($P=0.12$; Tabla 2). En contraste, el t_i en las mezclas de TS no mostró una

tendencia en particular y ΔG_c se incrementó siguiendo el orden TO>SBO>HOSfO. Esto indicó, que sobre condiciones similares de superenfriamiento, la TP requirió más energía para nuclear en el SBO y el HOSfO que en la TO, mientras que la nucleación de la TS requirió más energía de activación en la TO que en SBO y HOSfO.

Nuestros estudios previos que involucraron mediciones de anisotropía y simulación MM mostraron que la presencia de moléculas de TAGS conteniendo al menos una cadena de ácido palmítico en su estructura es un requisito para inducir una interacción molecular a través de fuerzas van der Waals (vdW) con la TP y TS y por lo tanto promover una estructuración previa a la nucleación de TAGS. De acuerdo a la composición de TAGS previamente reportada, el ácido palmítico no estuvo presente en la trioleína y sí en los aceites de soya y alto oleico como LLP, PLO, POL and, POO. Entonces, la presencia de TAGS conteniendo ácido palmítico en las mezclas hechas con HOSfO y SBO debería inducir una estructuración de TAGS previo a la nucleación. Comparando la ΔG_c para las mezclas hechas con TO, resulta un alto requerimiento de energía para la nucleación de la TP en las mezclas hechas con HOSfO y SBO. En contraste, la nucleación de la TS en HOSfO y SBO requirió menos energía que mezclada con TO.

Por otro lado, la organización de los TAGS en las mezclas investigadas pudo ser evaluada por la evolución de r_s mientras el sistema fue enfriado hasta alcanzar las condiciones isotérmicas para la cristalización. Así, la figura 2 muestra el comportamiento de la r_s de las mezclas en función del superenfriamiento durante la etapa de enfriamiento y el inicio de la cristalización isotérmica. Durante la etapa de enfriamiento, todas las mezclas investigadas mostraron un incremento exponencial de la r_s a medida que el superenfriamiento aumentó (Fig. 2). Sin embargo, se apreciaron los siguientes comportamientos. Todas las mezclas de TP observaron valores similares

de r_s y su cambio fue independiente del tipo de mezcla (ej., las tres mezclas presentaron la misma ecuación de ajuste). En el mismo contexto, los TAGS en el estado líquido fueron más estructurados en las mezclas de TP que en las mezclas TS (ej., todas las muestras de TS mostraron valores menores de r_s que las muestras de TP. Adicionalmente, en las mezclas de TS los valores de r_s mostraron el siguiente orden TO>HOSfO>SBO. Sin embargo, como se observó para ΔG_c, los valores de r_s para TS/SBO y TS/HOSfO no fueron estadísticamente diferentes.

Con lo anterior se concluye que durante la etapa de enfriamiento, la adición de TP a la TO, HOSfO y SBO no modifica la estructura original de los TAGS. En contraste, la adición de TS resulta en una disminución de la estructura de los TAGS líquidos particularmente en las mezclas TS/HOSfO y TS/SBO. Debido a que la mezcla TS/TO no mostró cambio en la pendiente (Fig. 2), la estructuración de los TAGS en la fase líquida no fue asociada a la trioleína.

De acuerdo al análisis de TAGS previamente reportado, el HOSfO presentó cerca de un 10% de TAGS conteniendo ácido palmítico en su composición mientras que el SBO tuvo aproximadamente 30% de estos TAGS. Entonces, los valores bajos de r_s observados en las muestras de TS en HOSfO y SBO comparados con los observados en TO, pueden estar asociados a la concentración de TAGS con ácido palmítico presentes en la mezcla.

La figura 3 muestra la evolución de la r_s bajo condiciones isotérmicas para diferentes mezclas de TP y TS. Una vez bajo estas condiciones, los valores de la r_s en todas las mezclas de TS permanecieron constantes en función del tiempo hasta que fue alcanzado el t_i. Este comportamiento fue observado a todas las T_{Cr} investigadas y, como ejemplo se muestra en la figura 3c la mezcla de TS/SBO cristalizada a 48 °C y 50 °C. El decremento en r_s observado

después de la nucleación (ej., después de t_i) fue asociado a la dispersión de la luz por los núcleos desarrollados. En contraste a las mezclas de TS, las mezclas TP/HOSfO y TP/SBO mostraron un incremento en la r_s en función del tiempo, este aumento fue en función directa del contenido de TAGS con al menos una cadena ácido palmítico y siguiendo el orden TP/SBO>TP/HOSfO. Así, las interacciones moleculares de la TP con POO, LLP y PLO/POL fueron tanto tiempo como temperatura dependientes y al parecer dificultaron la nucleación de la TP, ya que la ΔG_c fue mayor en las mezclas TP/SBO y TP/HOSfO comparada con la ΔG_c de la mezcla TP/TO (Tabla 2).

Otros investigadores han mostrado que mezclas de TP y POO cristalizan más lentamente que los TAGS puros, esto a pesar de que la TP y el POO observaron una cristalización independiente. Entonces, probablemente las interacciones moleculares que ocurrieron en la fase líquida entre la TP y los TAGS con ácido palmítico resultaron en el desarrollo de una estructura líquida lamelar de TAGS mixtos. Esto podría retrasar el proceso de nucleación hasta que la TP fuera segregada de dicha estructura lamelar. El resultado fue una mayor ΔG_c requerida para la nucleación de la TP en el sistema a medida que la concentración de TAGS con ácido palmítico (ej., LLP, POO y PLO/POL) aumenta en las mezclas.

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ORIGINAL ARTICLE

Pre-nucleation Structuring of Triacylglycerols and Its Effect on the Activation Energy of Nucleation

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Abstract Based in a previous study involving anisotropy measurements (r_s) and molecular mechanics simulations, we investigated the molecular interaction occurring among triacylglycerols (TAGS) before their nucleation, and its possible involvement in determining the activation free energy to develop a stable nucleus (ΔG_c). The Fisher-Turnbull equation was used to calculate the ΔG_c involved in the nucleation of tripalmitin (TP) or tristearin (TS) blended in a 25:75 ratio with triolein (TO), high oleic safflower oil (HOSfO), and soybean oil (SBO). The crystallization temperatures used were selected to obtain similar supercooling conditions in all blends investigated. As our previous study showed, the anisotropy measurements (r_s) showed that TAGS with at least one chain of palmitic acid (i.e., POO, LLP, PLO) induce TAGS' pre-nucleation structuring in both the TP and TS blends. However, for TS blends, the molecular interaction occurred well before attaining supercooling conditions required for crystallization (i.e., during the cooling stage). Once these supercooling conditions were achieved, TAGS with palmitic acid acted as template for TS crystallization. As a result, ΔG_c in TS/HOSfO and TS/SBO blends were lower than the ΔG_c in the TS/TO blend. In contrast, in the TP blends TAGS structuring (i.e., an increase in r_s) occurred as a function of time under isothermal conditions as a direct function of the concentration of TAGS containing at least one chain of palmitic acid. We postulate that the molecular interactions occurring in the liquid phase between TP and TAGS with palmitic acid, resulted in the development of a mixed TAGS lamellar liquid structure. This delayed TP nucleation until segregation of TP from the mixed TAGS lamellar liquid structure occurred. This resulted in a higher ΔG_c for TP nucleation as the concentration of LLP, POO, PLO, and PLO increased in the blends. The molecular interactions occurring among TAGS in the liquid phase under supercooling conditions must be understood, since they directly relate to the TAGS crystallization process that in turns determines the macroscopic properties evaluated by consumer in lipid-based products. The results presented in this manuscript are in this line of investigation.

Keywords Pre-nucleation structuring · Triacylglycerols structuring · Fisher-Turnbull · Anisotropy · Triacylglycerols nucleation

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Introduction

Vegetable oils and fats are multicomponent systems containing different families of triacylglycerols (TAGS). In these systems, the molecular relationships occurring among TAGS families determine the thermodynamic conditions (i.e., supercooling and supersaturation) that drive the formation of a solid from the liquid phase, the organization

of the solid phase, and its phase behavior. The resulting three-dimensional TAGS crystal network and the phase behavior of TAGS are major factors determining the physical and functional properties (i.e., rheology, liquid phase entrapment, mouthfeel, appearance, and spreadability) in products such as margarine, butter, confectionary coatings, and fillings[1]. Thus, the complex molecular interactions occurring among TAGS in the liquid phase under supercooling conditions must be first understood, since they directly relate to the TAGS crystallization process that in turns determines the macroscopic properties evaluated by consumer in vegetable oil and fat-based products.

Nematic[2], smectic[3], and discotic[4] phases have been proposed as the structural molecular arrangement for TAGS in the liquid phase. Although the actual structural mesophase organization of TAGS has not been established, all the work mentioned above agrees on the fact that TAGS molecules are somehow structured in a liquid crystalline-like state. Recently, we reported the pre-nucleation behavior of tripalmitin (TP) and tristearin (TS) in blends with triolein (TO), high oleic safflower oil (HOSfO), and soybean oil (SBO) as evaluated through anisotropy measurements and molecular mechanics simulations[5]. The TP/HOSfO and TP/SBO blends showed an increase in anisotropy, a behavior directly associated to an increase in the microviscosity of the blend that we interpreted as a structuring of TAGS before nucleation and growth of the crystals. A similar but less pronounced effect was also observed in the TS/SBO blends, but here the increase in anisotropy occurred after nucleation. This behavior was not observed in the TP/TO and the TS/TO blends[5]. We performed molecular mechanics simulations in an attempt to understand the molecular interactions responsible for this behavior. The simulation results indicated that the presence of TAGS molecules containing at least one chain of palmitic acid is a requisite to induce a molecular interaction through van der Waals forces with TP or TS, and therefore a pre-nucleation structuring of TAGS with the subsequent increase in the anisotropy, and therefore, in the microviscosity of the blends.

Within this framework, we investigated the molecular interaction occurring among TAGS before their nucleation, and its possible involvement in determining the activation free energy to develop a stable nucleus (ΔG_c). As in our previous study[5], the same TP and TS blends with TO, HOSfO, and SBO were used in the present investigation. The Fisher-Turnbull equation was utilized to calculate the ΔG_c using the induction time of crystallization (t_i) of the blends determined by differential scanning calorimetry (DSC) at different crystallization temperatures. To understand the behavior of ΔG_c in the blends, we performed an additional analysis of the anisotropy behavior in the blends to the one already done in our previous study[5]. The crystallization temperatures were selected in such a way to obtain similar supercooling conditions in all the blends investigated. As previously indicated[5], the blends investigated provided different degrees of molecular compatibility among the saturated TAGS (i.e., TP or TS) and the TAGS present in TO, HOSfO, and SBO. This since, in TO the major TAGS present was OOO (>99%), while in HOSfO were mainly OOO (≈66%), OOL (≈16%), POO (≈8.7%), and PLO (≈1.6%), and in SBO≈56% of TAGS were OOO, OOL, LLL, and LLO and ≈27% were POO, PLO, and LLP (where O=oleic acid, L=linoleic acid, and P=palmitic acid).

Materials and Methods

Blends Preparation

The TAGS, i.e., TP, TS, and TO, with purity higher than 99% were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The HOSfO and the SBO were obtained from local manufacturers, their main TAGS composition has been reported previously[5]. Blends of each of the saturated TAGS (i.e., TP and TS) in TO, HOSfO, and SBO were prepared in a 25:75 (wt/wt) ratio as previously described[5].

Isothermal Crystallization

Based on dynamic thermograms obtained by DSC with a TA Instruments Model Q2000 (TA Instruments, New Castle, Delaware, USA) as described previously[5], different crystallization temperatures were established for isothermal crystallization studies. Thus, blend samples (6–8 mg) sealed in aluminum pans were heated (80 °C/20 min) and then cooled to the corresponding crystallization temperature at 10 °C/min. For each crystallized blend the melting temperature at the peak (T_M') was calculated with the equipment software using the first derivative of the heat flux[6]. With the T_M' values, the equilibrium melting temperature ($T_M°$) for TP and TS in the blends was determined following the procedure of Hoffman and Weeks[7], as described by Perez-Martinez et al.[8] and Toro-Vazquez et al.[9]. Based on the $T_M°$ values, crystallization temperatures (T_{Cr}) were selected in such a way to obtain similar supercooling conditions for all TP and TS blends investigated. The supercooling was calculated as $T_M°-T_{Cr}$. At each T_{Cr}, the induction time of crystallization (t_i) was determined with the equipment software using the first derivative of the heat flux. For the TP blends, the t_i was measured from 36 °C to 41 °C every degree, and for the TS blends from 46 °C to 51 °C every degree. In all cases, at least three independent determinations were obtained. The results were analyzed through ANOVA and contrast among the

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treatment means using STATISTICA (V 9.0; StatSoft Inc., Tulsa, OK, USA).

Determination of ΔG_c

The Fisher-Turnbull equation was utilized to determine the magnitude of ΔG_c for the TP and the TS blends at each T_{Cr} investigated. The corresponding ΔG_c was calculated according to Ng[10] and Toro-Vazquez et al.[9] from the slope (s) of the linear regression of $\log[(t_i)/(T_{Cr})]$ vs. $1/T_{Cr}(T_M^\circ - T_{Cr})^2$ through the following equation:

$$\Delta G_c = sk/\left(T_M^\circ - T_{Cr}\right)^2$$

where, k is the Boltzmann constant. The results were statistically analyzed as in "Isothermal Crystallization" section.

Anisotropy Measurements

The anisotropy (r_s) of the blends was determined under the cooling and isothermal stages of the blends crystallization with a polarizer spectrophotometer (MD-5020 of Photon Technology International, London, ON, Canada) using the same time temperature as for t_i measurements (see "Isothermal Crystallization" section). We have described the details of the determination previously[5]. The r_s for the different TP and TS blends were plotted as a function of $T_M^\circ - T_{Cr}$.

Results and Discussion

t_i and ΔG_c Analysis

The T_M° for TP and TS in the three blends was 59.7°C (±0.83°C) and 70.2°C (±0.27°C), respectively (i.e., T_M° for TP and TS was independent of the type of blend). As stated in the "Material and Methods" section, the T_{Cr}s selected for the isothermal crystallization studies provided similar supercooling (i.e., $T_M^\circ - T_{Cr}$) conditions for TP and TS blends. Supercooling as calculated in the present manuscript, and supersaturation [Ln (β)] calculated as in our previous paper[5] (i.e., from the equality between the chemical potential equation and the Hildebrand equation), provided values of different magnitude but both measurements were highly correlated (Tables 1 and 2; $R^2>0.9830$, $P<0.001$).

The corresponding t_i and ΔG_c for the TP and TS blends as a function of supercooling are shown in Tables 1 and 2, respectively. At any given T_{Cr}, a single crystallization exotherm was always obtained that corresponded to the β polymorph for TP or TS crystallized in the blends (data not shown). X-ray analysis done to the blends[5] showed that at all T_{Cr}s investigated TP and TS crystallized in the β polymorph (i.e., all diffractograms showed the characteristic peaks at ≈20, ≈23, and ≈24 2θ corresponding to the 4.6, 3.85, and 3.70 Å short spacings of the β polymorph). The linear regression of $\log[(t_i)/(T_{Cr})]$ vs. $1/T_{Cr}(T_M^\circ - T_{Cr})^2$ provided in all cases determination coefficients (R^2) greater than 0.86 (i.e., $R>0.927$). As an example, Figure 1 includes

Table 1 Induction time by DSC (t_i, min) at different supercooling ($T_M^\circ - T_{Cr}$) used in the 25:75 (wt/wt) blends of tripalmitin (TP) or tristearin (TS) with triolein (TO), safflower oil high in triolein (HOSfO), or soybean oil (SBO)

$(T_M^\circ - T_{Cr})$ Ln (β)	TP[a]			$(T_M^\circ - T_{Cr})$ Ln (β)	TS[a]		
	TO	HOSfO	SBO		TO	HOSfO	SBO
23.7	2.04a	2.26a	2.08a	24.2	3.56a	4.76b	3.80a
0.26	0.04	0.09	0.05	0.24	0.47	0.25	0.72
22.7	2.50a	2.04a	2.85b	23.2	4.97a	5.08a	5.42a
0.24	0.08	0.14	0.47	0.22	0.45	0.20	0.13
21.7	2.66a	2.52a	3.30b	22.2	4.88a	5.73a	6.06b
0.22	0.10	0.31	0.05	0.21	0.27	0.35	0.28
20.7	2.61a	2.90b	3.70c	21.2	5.56a	5.42a	5.86a
0.21	0.20	0.00	0.25	0.19	0.06	1.33	0.48
19.7	2.98a	4.01b	4.48c	20.2	8.47a	6.64b	6.34b
0.19	0.15	0.19	0.63	0.17	0.34	0.34	0.06
18.7	4.79a	4.24a	5.09b	19.2	7.84a	8.14a	9.19b
0.18	0.62	0.18	0.61	0.17	1.24	0.82	0.21

The Ln (β) is the prevailing supersaturation as calculated by Dibildox et al.[2]

For the systems with TP or TS at the same $T_M^\circ - T_{Cr}$, values with a different letter are statistically different ($P<0.10$). Values with the same letter are statistically the same

[a] Values are shown as the mean and standard deviation of at least two independent measurements

t2.1 **Table 2** Free energy for nucleation (ΔG_c) at different supercooling ($T_M°-T_{Cr}$) used in the 25:75 (wt/wt) blends of tripalmitin (TP) or tristearin (TS) with triolein (TO), safflower oil high in triolein (HOSfO), or soybean oil (SBO)

t2.2	$(T_M°-T_{Cr})$ Ln (β)	TP[a]			$(T_M°-T_{Cr})$ Ln (β)	TS[a]		
t2.3		TO	HOSfO	SBO		TO	HOSfO	SBO
t2.4	23.7	1.07a	1.48b	1.64b	24.2	2.01a	1.19b	1.67b
t2.5	0.26	0.29	0.09	0.20	0.24	0.27	0.17	0.34
t2.6	22.7	1.16a	1.61b	1.79b	23.2	2.19a	1.30b	1.82b
t2.7	0.24	0.32	0.10	0.22	0.22	0.30	0.19	0.37
t2.8	21.7	1.27a	1.76b	1.96b	22.2	2.39a	1.42b	1.98b
t2.9	0.22	0.35	0.11	0.24	0.21	0.33	0.20	0.40
t2.10	20.7	1.40a	1.94b	2.15b	21.2	2.62a	1.55b	2.17b
t2.11	0.21	0.38	0.12	0.27	0.19	0.36	0.22	0.44
t2.12	19.7	1.54a	2.14b	2.38b	20.2	2.88a	1.71b	2.40b
t2.13	0.19	0.42	0.13	0.29	0.17	0.39	0.25	0.49
t2.14	18.7	1.71a	2.37b	2.64b	19.2	3.19a	1.89b	2.65b
t2.15	0.18	0.47	0.14	0.33	0.17	0.44	0.27	0.54

The Ln (β) is the prevailing supersaturation as calculated by Dibildox et al. [2]

[a] Values are shown as the mean and standard deviation of at least two independent measurements

For the systems with TP or TS at the same $T_M°-T_{Cr}$, values with a different letter are statistically different ($P<0.10$). Values with the same letter are statistically the same

Fisher-Turnbull plots for TP (Figure 1a) and TS (Figure 1b) blends, showing the corresponding R^2 and statistical significance of the regression equation. Thus, as has been shown by other authors [9-13], the Fisher–Turnbull equation, although originally developed for a single-component system, is applicable to complex TAGS systems as the TP and TS blends investigated.

Overall, for all blends investigated, t_i and ΔG_c increased as supercooling decreased (i.e., T_{Cr} increased). In general, for similar supercooling (and saturation), t_i was longer for TS blends than for TP blends. Thus, other factors besides supercooling must be considered to explain differences in the crystallization kinetics between homogenous saturated TAGS (i.e., TP vs. TS). For the TP blends and at all levels of supercooling investigated, t_i in the TP/SBO blend was the longest (Table 1) and ΔG_c increased following the order SBO>HOSfO>TO (Table 2). However, the ΔG_c for TP/SBO and TP/HOSfO blends were not statistically different at any of the T_{Cr} investigated ($P=0.25$; Table 2). In contrast, t_i in the TS blends did not show a particular trend and ΔG_c increased following the order TO>SBO> HOSfO. However, as observed in the TP blends, ΔG_c for TS/SBO and TS/HOSfO blends were not statistically different ($P=0.12$; Table 2). This indicated that under similar supercooling conditions, TP required more energy to nucleate in SBO and HOSfO than in TO, while TS nucleation required more activation energy in TO than in SBO and HOSfO. Within this framework, it is important to point out that in TO, the TS nucleation required approximately twice the activation energy than TP, while when blended with SBO and HOSfO, both TP and TS showed similar ΔG_c values.

Our previous study involving anisotropy and molecular mechanics simulations using the same blends[5] showed that the presence of TAGS molecules containing at least one chain of palmitic acid (i.e., POO) is a requisite to induce a molecular interaction through van der Waals forces with TP or TS, and therefore a pre-nucleation structuring of TAGS. According to the TAGS composition previously reported[5], no palmitic acid was present in TO, while palmitic acid was present in HOSfO and in SBO as LLP, PLO/POL, and POO. Then, the presence of TAGS containing palmitic acid in the blends made with HOSfO and SBO ought to induce a pre-nucleation structuring of TAGS. Comparing the ΔG_c for the blends made with TO, the TAGS structuring occurring before nucleation (i.e., in the liquid state) resulted in a higher energy requirement for TP nucleation in the blends made with HOSfO and SBO (Table 2). In contrast, TS nucleation in HOSfO and SBO required a lower ΔG_c than in the TS/TO blend.

Anisotropy Behavior under Cooling and Isothermal Conditions

The organization of TAGS in the blends might be evaluated by the evolution of r_s as the system is cooled to achieve isothermal conditions. The development and attainment of critical number of TAGS molecules structured in a liquid state or mesophase is a requirement to achieve TAGS nucleation. All the works associated with proposing molecular

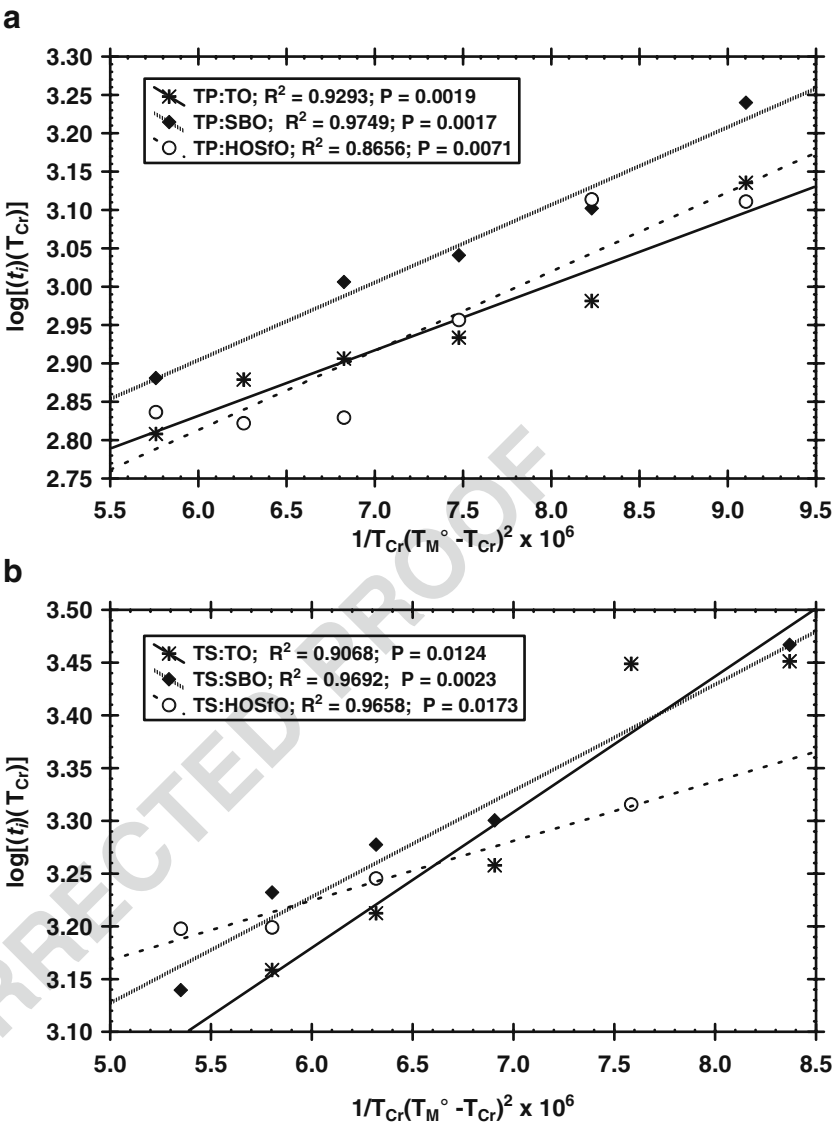

Fig. 1 Fisher-Turnbull plots for TP (**a**) and TS (**b**) blends with TO, HOSfO, and SBO. The legend shows the corresponding determination coefficient (R^2) and statistical significance (P) of the linear regression equation

arrangements for TAGS melts agrees on this[2-4, 14-17]. In fact, the change in TAGS viscosity with temperature is determined by the shape and size of the TAGS liquid structures[18] that is indirectly measured by r_s. Within this framework, in an attempt to understand the behavior of ΔG_c in the blends, Figure 2 shows the behavior of r_s in the blends as a function of supercooling during the cooling and isothermal stages. The r_s value for the isothermal stage plotted in Figure 3 was obtained once T_{Cr} was attained and corresponds to the average of r_s values obtained during the first 60 s under isothermal conditions (i.e., before t_i was achieved in the blend).

During the cooling stage, all blends investigated showed an exponential increase of r_s (i.e., TAGS achieved a higher level of liquid structure) as supercooling increased (R^2>0.95; Figure 2). Nevertheless, TP and TS blends showed some distinctive features of how r_s changed as a function of supercooling. Thus, all TP blends observed similar r_s values and its change as a function of supercooling was independent of the type of blend [i.e., the same equation fitted the r_s vs. ($T_M°-T_{Cr}$) data for the three TP blends; $r_s=0.0236\times\exp[0.0331\times(T_{Cr}-T_M°)]$, $R^2=0.9914$]. In the same way, TAGS in the liquid state were more structured in the TP blends than in the TS blends (i.e., overall TS blends showed lower r_s values than the TP blends). Additionally, in the TS blends the r_s values showed the following order TO>HOSfO>SBO. However, as observed for ΔG_c, the r_s values for TS/SBO and TS/HOSfO blends were not statistically different.

From the above, we conclude that during the cooling stage, the addition of TP to TO, HOSfO, and SBO did not modify the original TAGS liquid structure. Such behavior was followed for all TP blends at the different T_{Cr}s used for isothermal crystallization (Figure 2). In contrast, the

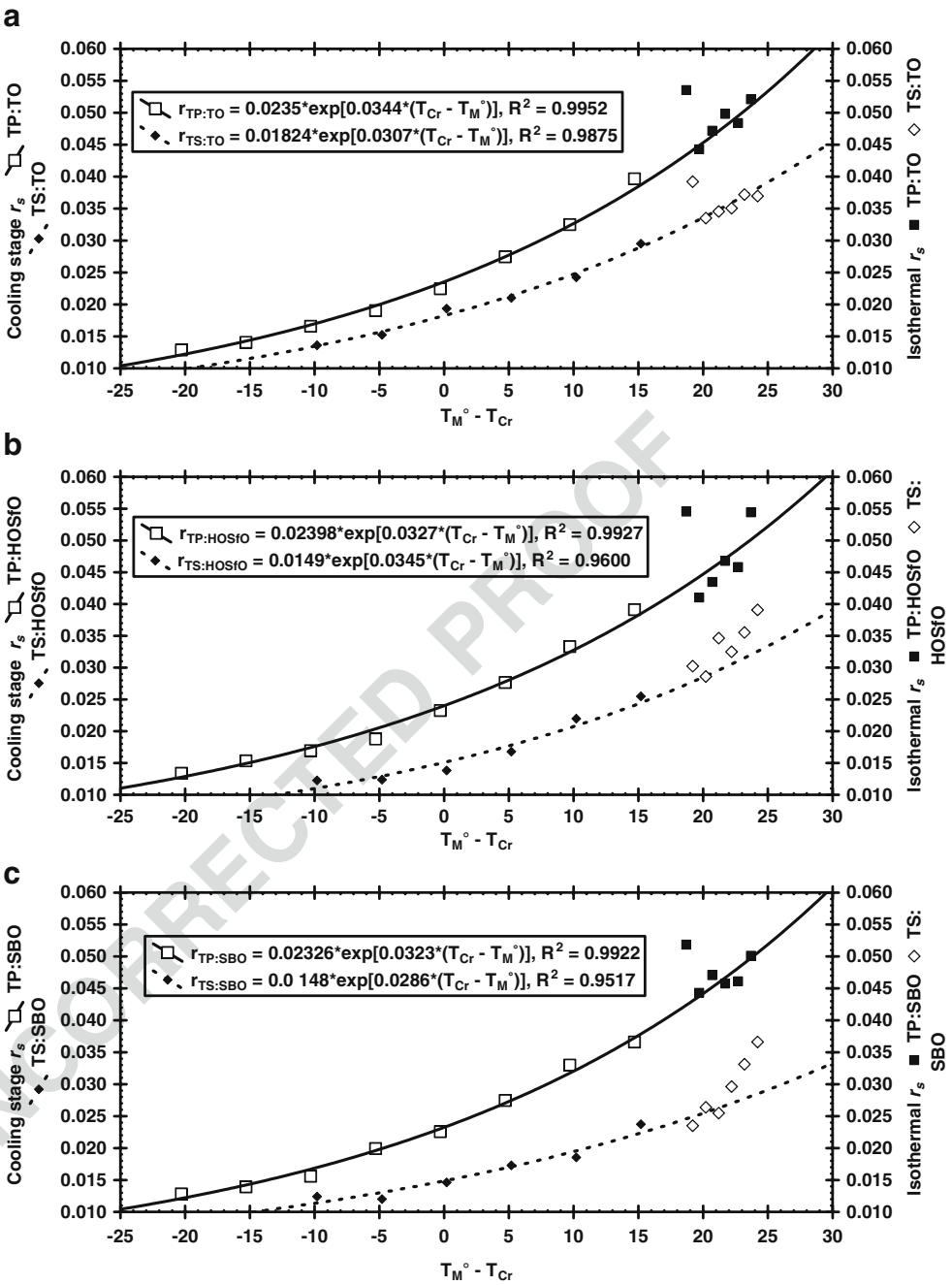

Fig. 2 r_s as a function of supercooling for the TP and TS blends with TO (**a**), HOSfO (**b**), and SBO (**c**) during the cooling stage and at the T_{Cr} used for isothermal crystallization. The fitting equations show considered r_s values just during the cooling stage

addition of TS resulted in decreasing the TAGS liquid structure particularly in the TS/HOSfO and TS/SBO blends. Additionally, except for the TS/TO blend, r_s in the TS/HOSfO and TS/SBO blends showed a significant change in the slope once isothermal conditions were achieved (Figure 2). This change in the behavior of r_s at such supercooling conditions (i.e., T_{Cr}s ≤51 °C), was associated with the developing of a higher TAGS structural organization than the one anticipated by the exponential fitting of r_s during the cooling stage (Figure 2). Thus, before t_i was achieved TAGS in the TS/HOSfO and TS/SBO blends accomplished a particular structural organization. Since the TS/TO blend did not show this change in the slope (Figure 2), TAGS structuring in the liquid state was not associated to triolein.

According to the TAGS analysis previously reported[5], HOSfO had about 10% of TAGS containing palmitic acid in their composition whereas SBO had approximately 30% of these TAGS. Thus, the lower r_s values observed in the TS/HOSfO and TS/SBO blends in comparison with the ones observed in the TS/TO blend and the extent of change in the slope observed in the TS blends (Figure 2), might be associated with the concentration of TAGS containing palmitic acid present in the blend. Thus, a more pronounced slope change was observed in the TS/SBO blend than in the

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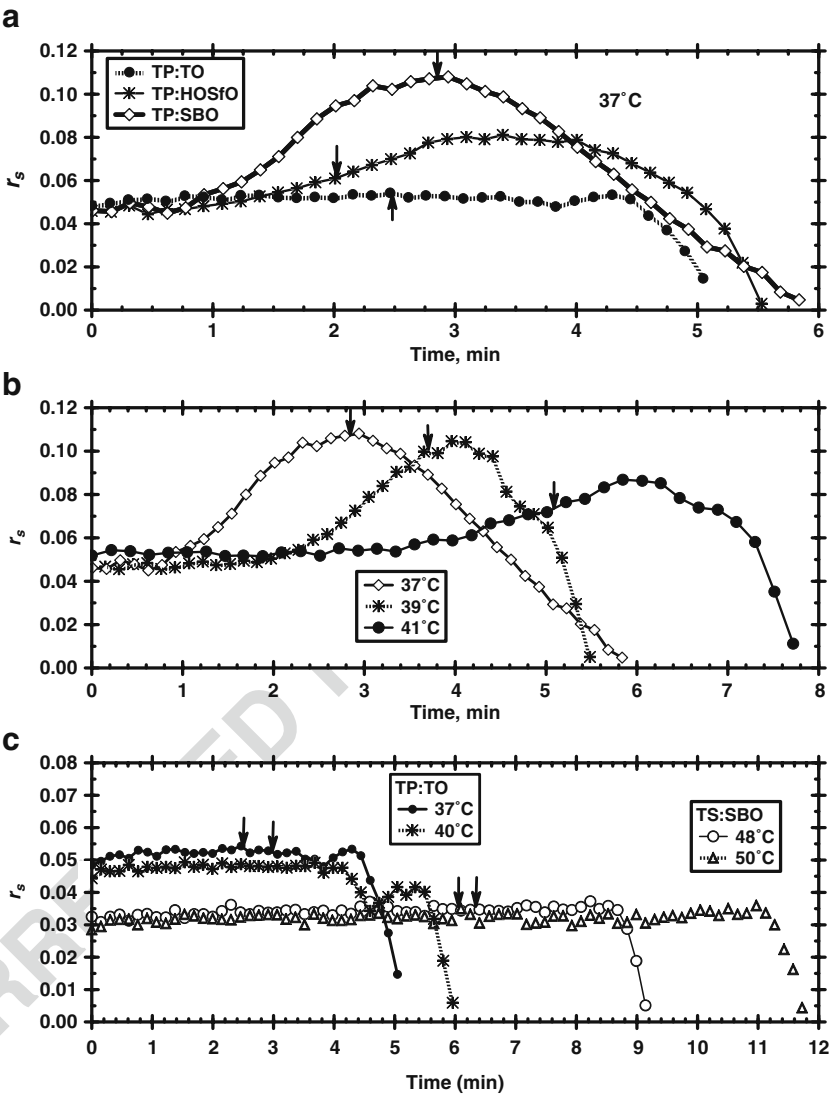

Fig. 3 Evolution of r_s under isothermal conditions for TP/TO, TP/HOSfO, and TP/SBO blends at a T_{Cr}=37°C (**a**), TP/SBO blends (**b**), TP/TO and TS/SBO blends (**c**) at different T_{Cr}. The *arrows* indicate the induction time for crystallization (t_i) for the corresponding blends

TS/HOSfO, and no change was observed in the TS/TO blend. The molecular interaction of TS with POO, LLP, and PLO/POL seems to occur well before achieving supercooling conditions that resulted in TAGS crystallization (i.e., during the cooling stage). TO, HOSfO, and SBO do not contain TAGS with stearic acid, and since stearic acid has a longer hydrocarbon chain length than palmitic acid, structural compatibility between TAGS containing palmitic acid and TS was not effective. The result was a decrease in TAGS liquid structure and therefore, lowers r_s values in the TS/HOSfO and TS/SBO blends. Once supercooling conditions required for TAGS crystallization were prevalent (i.e., T_{Cr}s≤51°C), palmitic acid present in the TAGS facilitated TS nucleation. As a result, ΔG_c was lower in the TS/HOSfO and TS/SBO blends compared with the activation energy required by TS in the TS/TO blend (Table 2). Probably, under such supercooling conditions, TAGS with palmitic acid acted as template for TS structuring in the liquid state and from here the change in the slope of the r_s vs. (T_M°−T_{Cr}) relationship in the TS/SBO and TS/HOSfO blends (Figure 2). The result was a lower ΔG_c for TS crystallization in SBO and HOSfO, in comparison with the ΔG_c observed in the TS/TO blend (Table 2).

Once under isothermal conditions, the r_s values in all TS blends remained constant as a function of time until t_i was achieved (i.e., no solid phase was present). This behavior was observed at all T_{Cr}s investigated. Examples of such behavior are presented for the TS/SBO blend at the T_{Cr} of 48 °C and 50 °C in Figure 3c. The decrease in r_s observed after nucleation (i.e., after t_i; Figure 3) was associated to light scattering from the nuclei developed. In some cases (i.e., TS/TO at 49 °C and 50 °C and TP/TO at 39 °C and 40 °C; results not shown), after t_i, we observed an increase in r_s before decreasing. In our previous paper [5], we associate this behavior as a structuring of TAGS due to molecular interaction between TS and TAGS containing

palmitic acid. However, since this r_s peak occurred right after nucleation, the behavior might be associated to remelting of the solid phase due to the heat generated during TAGS crystallization with the subsequent increase in r_s. As crystallization continued and more solid phase was present, light scattering produced a decrease in r_s.

In contrast to the TS blends, the TP/SBO and TP/HOSfO blends showed an increase in r_s as a function of time under isothermal conditions. The increase in r_s (i.e., TAGS structuring) was a direct function of the concentration of TAGS containing at least one chain of palmitic acid, i.e., the increase in r_s followed the order TP/SBO>TP/HOSfO and no increase in the TP/TO blend (Figure 3a). For the TP/HOSfO (data not shown) and the TP/SBO (Figure 3b) blends, the r_s onset was an inverse function of supercooling, and the extent of its increment was a direct function of supercooling. Thus, the molecular interaction of TP with POO, LLP, and PLO/POL were time and temperature dependent. Such molecular interactions seem to make TP nucleation more difficult in the TP/SBO and TP/HOSfO blends than in the TP/TO blend. As a result ΔG_c was higher in TS/SBO and TS/HOSfO blends when compared with the ΔG_c in the TP/TO blend (Table 2). Mihara et al.[19] showed that mixtures of TP and POO crystallize more slowly that the pure TAGS. This regardless an independent crystallization of TP and POO was observed in the mixture (i.e., no mixed crystal formation occurred)[19]. Probably, the molecular interactions occurring in the liquid phase between TP and TAGS with palmitic acid resulted in the development of a mixed TAGS lamellar liquid structure. This would delay the nucleation of TP, until segregation of TP from the mixed TAGS lamellar liquid structure occurred. The result was a higher ΔG_c for TP nucleation as the concentration of TAGS with palmitic acid (i.e., LLP, POO, PLO, and PLO) increased in the blends (Table 2).

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IV. CONCLUSIONES GENERALES

CONCLUSIONES GENERALES

En conclusión, este trabajo estudió el ordenamiento previo a la nucleación que ocurre en mezclas de TAGS saturados con diferentes aceites vegetales líquidos. Los experimentos por espectroscopia de fluorescencia polarizada dieron una visión sobre la estructuración a nivel microscópico que ocurre en esta etapa. Para aclarar el tipo de interacciones responsables de este efecto a nivel molecular, la simulación MM resultó ser una herramienta rápida y simple para abordar este aspecto y puede proporcionar información complementaria a los datos experimentales, haciendo notar que los resultados obtenidos necesitan ser cuidadosamente interpretados según las condiciones usadas en las simulaciones.

Los resultados obtenidos en esta investigación demostraron que:

- La anisotropía y la viscosidad varían linealmente, de aquí que la anisotropía, en el rango estudiado, es una medida de la microviscosidad del sistema.
- Es posible evaluar por medio de la anisotropía los cambios en la microviscosidad de la fase líquida como efecto de interacciones moleculares que conducen a la estructuración de la mezcla previa a la nucleación y crecimiento de los cristales en sistemas complejos.
- Son las fuerzas van der Waals las responsables del comportamiento líquido o sólido de los TAGS y por lo tanto, piezas clave causantes del aumento en la microviscosidad de un sistema de TAGS previo a su nucleación.
- Ocurre un comportamiento específico en función de la composición molecular de un TAG en particular. Así, la presencia de moléculas que contienen al menos una cadena de

ácido palmítico en su composición de TAGS, induce a un aumento en la microviscosidad de la mezclas previo a la nucleación y es por lo tanto requisito para inducir una interacción molecular.

- La ΔG_c requerida para formar núcleos estables de un TAG saturado (ej., TP) es mayor a medida que la concentración de TAGS con ácido palmítico (ej., LLP, POO y PLO/POL) aumenta en las mezclas.

Aunado a lo anterior, en general se logró una mejor comprensión de las interacciones moleculares responsables de un fenómeno observado experimentalmente. Además, es nuestra expectativa el que este trabajo haya establecido las bases para la aplicación de simulaciones MM en sistemas similares a los aquí presentados.