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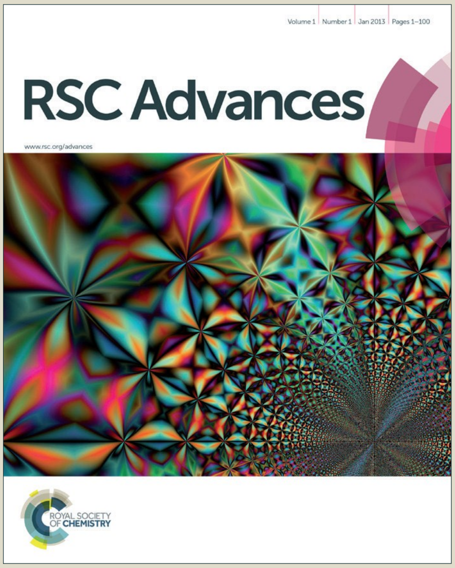

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Engineering the viscosity and melting behaviour of triacylglycerol biolubricants via interesterification

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The effects of blending, chemical and enzymatic esterification on the viscosity, melting and crystallization behaviour of blends of high oleic algal oil (HOAO), tricaproin (trihexanoin) and tricaprylin (trioctanoin) were determined. Blending HOAO with lower molecular weight tricaproin and tricapryllin reduced viscosity in a predictable fashion related to the average molecular weight of the molecular mixtures. For example, the HOAO kinematic viscosity at 40°C decreased from 39.6cSt to 33.3cSt upon addition of 30% (mol/mol) tricaprylin. However, molecular randomization by interesterification did not affect the kinematic and dynamic viscosities of the blends; the viscosity of the 30:70 HOAO:tricaprylin blend decreased non-significantly to 31.4 cSt. On the other hand, addition of tricaprylin and tricaproin combined with chemical and enzymatic interesterification did significantly reduce the crystallization and melting temperatures of the mixtures. The melting temperatures of HOAO decreased from -3.7°C to -4.5°C upon addition of 30% (mol/mol) tricaprylin, while the crystallization temperature decreased from -34.8°C to -37.8°C. Surprisingly, enzymatic interesterification of this mixture drastically decreased the crystallization and melting temperatures of the blends, to -58.3°C and -21.4°C, respectively. A statistical thermodynamic model was developed to explain the decrease in melting temperature as a function of molecular randomization of the mixtures, which predicted the observed decrease quantitatively. This decrease in melting temperature was governed by an enthalpic effect. Thus, viscosity reduction in triacylglycerol mixtures is solely affected by the average molecular weight of the constituent triacylglycerol molecules, but their melting points can be significantly reduced by randomizing chemical structure. Blending and interesterification are both required to reduce viscosity and decrease the melting point of triacylglycerol biolubricants.

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Introduction

Vegetable oils have been considered as replacements for mineral and synthetic oils in lubricants[1]. Vegetable oils are biodegradable and non-toxic, have high lubricity, good anticorrosion properties, a relatively high viscosity index (*i.e.*, relatively small changes in viscosity with temperature), a high boiling point and flash-point, and low volatility.[2] However, there are some serious drawbacks that have prevented the widespread use of these biodegradable stocks in lubricant formulations. Most vegetable oils are much less stable than mineral and synthetic oils. Triacylglycerols (TAGs), the main molecular species in oils, are labile to hydrolysis in the presence of water and heat, and can be oxidatively unstable since they contain labile double bonds in the fatty acid chains. This, however, will depend on the source of the oils, since some are more unsaturated than others. Since unsaturated double bonds are active sites for oxidation reactions, the kinetics of oil oxidation will depend on the number of double bonds present in the TAG molecules.[8] Moreover, Fox and Stachowiak demonstrated a close relationship between increasing levels of hydroperoxides in oxidized vegetable oil lubricants and increased wear.[9] The oxidation issue can be partially solved by using saturated and/or mono-unsaturated fatty acids (high oleic acid (*cis*-9-18:1) containing oils). Oleic acid is quite stable towards oxidation.[3] However, there are other issues related to the use of vegetable oils. Their viscosity, crystallization temperatures and melting points are generally higher than those of synthetic and mineral oils, thus limiting the range of applications significantly. In order to be able to use vegetable oils as lubricants widely, they need to be structurally modified.[1]

Viscosity is a measure of internal friction between molecules, and thus, the viscosity of oils depends mainly on the average molecular weight of oils, *i.e.*, the fatty acid composition, but can depend on the presence of functional groups. For example, hydroxyl groups, present in the main fatty acid of Castor oil, ricinoleic acid, interact with other hydroxyl groups via hydrogen bonding, leading to an increased viscosity of this oil. However, the nature of the fatty acid chains esterified onto the glycerol backbone of TAGs will also play a role. For example, oil viscosity has been shown to be positively correlated to the amounts of oleic acid present and negatively correlated to the amount of PUFAs.[4] In general, the viscosity of vegetable oils increases with increasing chain length of their constituent fatty acids. Geller and Goodrum[5] showed that the dynamic viscosity of pure saturated fatty acids (C6:0 to C18:0) is correlated to their carbon number in a second order polynomial fashion at 75°C. The number of double bonds and fatty acids isomer configuration (*cis* or *trans*) also had an influence on viscosity.[5] Viscosity is a temperature dependent physical property and generally decreases exponentially with increases in temperature.[6,7]

Saturated fatty acids will tend to crystallize at relatively high temperatures and thus cause a drastic increase in viscosity, which will eventually end in a crystallized lubricant, which is not desirable. Moreover, other factors such as the presence of minor components, phospholipids for example, will affect the viscosity and stability as well. Purity is an important factor when considering these materials. Our recommendation is to use the highest purity possible. This should not be a problem since most edible oils are refined, bleached and deodorized (RBD); it is common practice.

Low molecular weight saturated triacylglycerols, *i.e.*, tricaproin and tricaprylin, have high oxidative stability, which makes them suitable materials as components for biolubricant manufacture compared to regular vegetable oils containing polyunsaturated fatty acids (PUFAs). Vegetable oils such as Cuphea oil which contain mostly tricaprylin and tricaprin might be suitable for direct use as lubricants due to their low viscosity and high stability.[1] However, because of a shortage of these natural oils, an alternative approach could be the modification of vegetable oils by incorporation of medium chain fatty acids via interesterification. Although the formulation of bio-lubricants with a high percentage of unsaturated fatty acids will result in a lower viscosity and better cold flow properties (lower crystallization temperature and melting point), their oxidative stability is low.

Therefore an optimum balance has to be striken between fatty acid chain length and the degree of unsaturation to formulate a viable biolubricant with high oxidative stability, low viscosity and low crystallization temperature.[10] In the oleochemical industry, some processes such as alkylation, arylation, cyclization, hydrogenation, and epoxidation are used to modify unsaturated fatty acids in TAGs, and thus the viscosity and thermo-oxidative stability of the oil.[2] For example, during partial hydrogenation of vegetable oils, viscosity, crystallization temperature, melting points and oxidative stability increase as a result of increasing the saturation of fatty acids as well as their partial geometrical isomerization (*trans* fatty acid formation).

Interesterification (chemical or enzymatic) is another chemical modification method used to modify the fatty acid distribution within TAG molecules.[11] Interesterification can be used to alter the physical properties of oils, and improve their oxidative stability. Thus, blending high oleic algal oil with low molecular weight triacylglycerols (tricaproin and tricaprylin), combined with interesterification may be a suitable strategy to make high oxidative stability, low viscosity, and low dropping point biolubricants. Here we report on the effects of chemical and enzymatic esterification on the viscosity, melting point and crystallization temperature of blends of high oleic algal oil with tricaproin and tricaprylin.

Experimental

Materials and methods

Refined bleached and deodorized high oleic algal oil (HOAO) was a gift from Solazyme Inc. (South San Francisco, CA, USA) Tricaproin (>95.0%) and tricaprylin (>93.0%) were purchased from TCI (Tokyo, Japan) and Sigma-Aldrich (Oakville, Canada), respectively. Based on gas-liquid chromatographic analysis, the fatty acid composition of HOAO was myristic acid 0.8%, palmitic acid 9.3%, stearic acid 2%, oleic acid 87.4%, and linoleic acid 0.7%. Sodium methoxide was purchased from Sigma-Aldrich (Oakville, Canada) and Trisyl Silica from Grace Co. (Maryland, USA). Lipozyme TL IM (1,3-specific immobilized *Thermomyces Lanuginosus* lipase) was purchased from Sigma-Aldrich (Oakville, Canada).

Blending and interesterification procedures

Blending

The 30:70 and 50:50 (mol/mol) blends of tricaproin or tricaprylin to HOAO were prepared based on their fatty acid-weighted molecular weight ($\overline{MW} = \sum_{i=1}^{n} x_i MW_i$), where x_i represents the weight fraction of the i^{th} fatty acid of i^{th} molecular weight.

Chemical interesterification

The interesterification reactions were performed first by heating oils to 95±2ºC to dry the samples under vacuum for 20 minutes. Chemical interesterification was started by adding 0.5% (w/w) sodium methoxide during stirring at 95±2 ºC for 30 minutes under vacuum (50 mmHg). To stop the reaction and inactivate the catalyst, 2% w/w aqueous citric acid solution (from 20% solution stock) was mixed in for 5 minutes followed by addition of 1% Trisyl silica (w/w) to remove soap and other impurities. After centrifugation, the clear oil was dried at 90^{o}C under vacuum (50 mmHg).

Enzymatic interesterification

The reaction mixtures consisting of 30:70 and 50:50 molar ratios of tricaproin or tricaprylin to HOAO were transferred to an Erlenmeyer flask. Lipozyme TL IM (Novozymes, Franklinton, USA) (10% w/w) was added to the reaction mixtures. The Erlenmeyer flask was placed in a shaking water bath at 50±1 ºC for 24 hours at 125 rpm. The reaction was stopped after addition of a mixture of acetone and ethanol (1:1, v/v). Free fatty acids were neutralized with a 0.5 M NaOH followed by addition of 1% Trisyl (w/w) to remove soap and other impurities. Finally products were centrifuged. After centrifugation, the clear oil was dried under vacuum.

Viscosity measurements

The rheology of the samples was studied using an Anton Paar MCR302 rheometer (Ashland, VA, USA) with a concentric cylinder geometry (CC17). The viscosity of the samples was obtained while controlling the temperature using peltier concentric cylinder thermal unit at 40°C. The analyses were carried out using shear rates varying from 1.0 to 100s^{-1} in three replicates.

Viscosity of blends calculation

The viscosity of the blends was calculated using the Refutas equation.[12] Calculating the viscosity blending index of a sample consisting of two oils having different viscosities involves two steps. The first step involves calculation of the Viscosity Blending Index (VBI) of each component of the blend using equation 1,

$$VBI = 14.534 \times \ln[\ln(v + 0.8)] + 10.975, \qquad (1)$$

where v is the kinematic viscosity in centistokes (cSt)

The second step is the calculation of VBI of the blends (VBI_{Blend}) using equation 2,

$$VBN_{Blend} = \sum_{i=1}^{n} x_i VBI_i \, , \qquad (2)$$

where x_i is the mass fraction of each component in the blend.

Finally, the kinematic viscosity of blends (cSt) was calculated using equation 3.

$$V = \exp(\exp(VBN_{\mathrm{Blend}} - 10.975) \div 14.534) - 0.8 \qquad (3)$$

Density measurement

Mass densities of all the samples were measured by means of a pycnometer at 40 ^{o}C.[13] First a 50-ml Pyrex pycnometer with a close-fitting ground glass stopper with a capillary hole through it was calibrated with distilled water at 40^{o}C. For each analysis, the pycnometer was filled up with the sample and then placed in an oven at 40^{o}C for 45 minutes. The excess oil was then removed from the pycnometer and glass stopper with the aid of a tissue paper. The pycnometer was weighted rapidly to minimize temperature drop.

Thermal analysis by Differential Scanning Calorimetry

The crystallization temperatures of blended and esterified samples were determined using a TA Instruments Q2000 differential scanning calorimeter (Mississauga, ON, Canada). Nitrogen was used as the purge gas with the flow of 18 mL/min. Oil samples of 8–15 mg were weighed into aluminum pans and lids were hermetically sealed into place. An empty, hermetically sealed aluminum pan was used as reference. In this experiment, samples were submitted to cooling from 30 °C to –60 °C at 5°C/min and held for 5 minutes at –60°C. Melting points of samples were determined by heating from –60 °C to 30 °C at 5°C/min. The peak of crystallization and melting curves were considered as crystallization and melting temperatures.

Fatty acid composition analysis by Gas Liquid Chromatography

The fatty acid composition of HOAO was determined using Gas Liquid Chromatography (GLC). The HOAO underwent a transmethylation reaction as described by Christie.[14] Fatty acid methyl esters (FAME) were determined using a capillary GLC equipped with a 60m BPX70 column of 0.22mm internal diameter with 0.25mm film thickness (SGE Inc., Austin, TX, USA). An Agilent 6890-series Gas Chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) with a 7683-series autosampler were used to house the column. The oven temperature was programmed to increase from 110 to 230°C at a rate of 4°C/min and maintained at 230°C for 10 min. The injector and detector temperatures were 240 and 280°C, respectively. Hydrogen was used as the carrier gas at an average velocity of 25 cm/s. Peaks were identified by comparison to FAME standards.

Triacylglycerol profile analysis by LC/MS

Samples were prepared by dissolving the oil to a concentration of 5 mg/mL in toluene/2-propanol (3:1) and analyzed on a Shimadzu LC/MS (Columbia, MD, USA) system consisting of a Nexera UHPLC coupled to an 8030 triple quadrupole mass spectrometer equipped with an APCI source. A Shimadzu Shim-pack XR-ODS III (2.2 μm, 2.0 x 200 mm) column at 30 ^{o}C was used, eluting with a gradient of acetonitrile (A) and dichloromethane/2-propanol (1:1, v/v) (B) at 0.48 mL/min. The linear gradient went from 30 to 51% B over 27 min, then to 74% B over 3 min and held there for an additional 3 min. Positive ion mass spectra were recorded as Q3 scans, from m/z 350 to 1050 at a scan rate of 1428 u/sec. Argon was used as the collision gas at 230 kPa.

The APCI source temperature, heat block, and desolvation line were at 300, 200, and 250 °C, respectively, and nitrogen was used as the nebulizing and drying gas, with flows set to 3 and 5 L/min, respectively.

Triacylglycerol sn-2 profiles

TAGs were isolated from total lipid on Agilent (Varian) Bond Elut aminopropyl solid phase extraction cartridges, as described by Kaluzny.[15] Deacylation of the TAGs was performed similarly as described by Rauf et al.[16], at one-tenth the scale. Briefly, 10 mg of purified TAGs were added to 300 μL of 1 M tris-hydroxymethylaminomethane buffer, pH 8, followed by 2 mg of porcine pancreatic lipase (Sigma-Aldrich). The mixture was agitated vigorously, and then 50 μL bile salt solution (1 g/L) and 20 μL calcium chloride solution (2.2% w/v) were added. The mixture was held at 40 °C for 3 min with intermittent agitation. The reaction was quenched by placing the reaction tube in cool water and adding 100 μL of 6 M HCl. The reaction mixture was extracted with diethyl ether (3 x 1 mL), the extracts were combined, washed with water, and dried over anhydrous sodium sulfate. The ether was removed with a stream of nitrogen, and the monoaclyglycerols (MAGs) were isolated by solid phase extraction, on aminopropyl cartridges, as described before.[5] The isolated MAGs were subjected to standard direct transesterification to provide the sn-2 profiles.

Statistical analysis

All analyses were run in triplicate and all results were reported as mean values ± SD. The statistical analysis was carried out using GraphPad's Prism software (La Jolla, CA, USA), version 6.0. A one-way ANOVA and Tukey's test were used to evaluate significant differences between NIE, CIE and EIE oil mixtures in terms of kinematic viscosity, crystallization and melting temperatures.

Results and discussion

The triacylglycerol profiles and corresponding molecular weights of blended (NIE), chemically esterified (CIE) and enzymatically esterified (EIE) mixtures of HOAO with ticaproin and tricaprylin used in this study are presented in Tables 1 and 2. The main TAGs in both blended mixtures (30:70 and 50:50 mol/mol ratios) were OOO, OOP, CoCoCo and CyCyCy, which made up more than 90% of total TAGs in each blend. Based on TAG composition, it is clear that each mixture has high oxidative stability due to their high oleic acid and medium chain saturated fatty acids content. After chemical and enzymatic interesterification, TAG composition changed and CoOO, CoOCo, CyOO and CyOCy became the major TAGs present.

INSERT TABLES 1 AND 2 HERE

There was no significant difference in kinematic viscosity at 40°C of 100% HOAO before and after interesterification (Table 3). This suggests that random and/or specific rearrangement of fatty acids within an oil does not have any effect on its dynamic viscosity. Dynamic and kinematic viscosities of the 30:70 and 50:50 (mol/mol) mixtures at 40 °C are presented in Figures 1 and Table 3, respectively. There was no significant difference between the dynamic viscosities of non-interesterified and interesterified oils at 40°C.

INSERT TABLE 3 AND FIGURE 1 HERE

The density and viscosity of vegetable oils depends on the fatty acid chain length and the amount of unsaturated fatty acids of their constituent TAGs.[6,17] Since during interesterification of HOAO alone, the average fatty acid composition (chain length and unsaturation) did not change, the viscosity also remained practically unchanged before and after interesterification (Table 3). The density of different oils, in conjunction with the dynamic viscosity at 40 °C was used to calculate the kinematic viscosity at that temperature (Table 3). A greater addition of medium chain fatty acids to the HOAO blends (50:50 vs 30:70) lead to a greater viscosity reduction, and the viscosity reduction was greater for blends containing the lower molecular weight tricaproin. TAG molecular weight had a strong influence on viscosity, as previously reported.[18] At 40°C, a 50:50 ratio of enzymatically interesterified tricaproin:HOAO mixture had a kinematic viscosity of 23.2 cSt compared to 29.5 cSt for the 30:70 ratio. Based on results presented in Table 3, the kinematic viscosity of the oils was also correlated to the average molecular weight of TAG molecules. However, in contrast to the results for dynamic viscosity, we did observe a very small, but significant, decrease in viscosity upon enzymatic 1,3-specific randomization of fatty acids on the glycerol backbone (Table 3). For example, in tricaproin/HOAO mixtures, formation of new medium chain TAGs (CoOCo and CoOO) and reduction in OOO after enzymatic esterification was greater than for chemical interesterification in both 30:70 and 50:50 mixtures. Maybe the enrichment in these TAGs was responsible for the small reduction in kinematic viscosity observed.

The higher crystallization temperature of saturated TAGs can create difficulties in low temperature applications as result of partial or full crystallization of the oil. Decreasing the crystallization temperature, or pour point, of oils is necessary for bio-lubricants to have functionality at low temperatures.[19] Differential Scanning Calorimetry is an efficient method for characterizing the melting and crystallization behavior of lubricants, as it is more accurate than classical pour point measurements.[16,20]

INSERT TABLE 4 HERE

The crystallization temperature depends on molecular interactions between TAG molecules in the solid state.[21] Linear saturated fatty acids pack efficiently into a crystalline lattice while unsaturated fatty acids pack much less efficiently due to the kinked geometry of the *cis* fatty acid chain. Since interesterification of 100% HOAO did not change the average molecular weight of TAG molecules (chain length and unsaturation), the crystallization and melting temperatures remained unchanged before and after interesterification (Table 4). The incorporation of medium chain fatty acids into HOAO through interesterification reactions lead to a significant drop in crystallization temperature, melting temperature, and both crystallization and melting enthalpies of the mixtures (Table 4). For example, after enzymatic esterification of a 50:50 mixture of tricaproin/HOAO, the crystallization temperature decreased to -74^{o}C compared to -42^{o}C for its NIE counterpart. In Figure 2, differential scanning calorimetric traces of 50:50 (mol/mol) tricaprylin:HOAO before and after chemical interesterification are shown. The crystallization and melting temperatures for the mixtures were -38.9^{o}C and -5.7 ^{o}C, respectively while after chemical interesterification these values decreased to -49.6^{o}C and -21.7^{o}C, respectively.

INSERT FIGURE 2 HERE

An explanation for the observed decrease in melting point of the interesterified mixtures will be attempted below.

The free energy change is a function of enthalpy and entropy changes, namely $\partial G = \partial H - T\partial S$. A reaction will be spontaneous when $\partial G = 0$. Thus for the melting phase transition, the melting temperature, T_m, can be expressed as $T_m = \left(\dfrac{\partial H_m}{\partial S_m}\right)_P$. Thus, both the enthalpy and entropy of melting can affect the ultimate melting temperature of the solid. We shall consider the effects of molecular randomization on the enthalpy of melting.

Consider two TAG crystals composed of long-chain (A) and short-chain (B) hydrocarbon chains. Let the chains be of length L_A and L_B with $L_A \gg L_B$. In their crystalline states, if the chains are packed approximately in a triangular lattice, then the pairwise van der Waals interaction energy per parallel all-*trans* hydrocarbon chain are, approximately $-6AL_A$ and $-6AL_B$, respectively.[22] Here A is proportional to the Hamaker coefficient. We call this the "pure" case. Although the demonstration that the interaction energy is proportional to the length of the chains has been calculated for a pair of long parallel all-trans chains, the scaling for chains with only one double bond is likely to be correct.

The total energy for N_A and N_B such hydrocarbon chains is then, approximately,

$$E_{pure} = -3AL_AN_A - 3AL_BN_B, \tag{4}$$

where the factor of 3 indicates that we have avoided double counting. Now consider randomly interesterified TAG molecules in which the average ratio of A:B chains per molecule is $N_A : N_B$, and consider Figure 3. Let us assume that these molecules crystallize approximately so that the chains occupy the sites of a triangular lattice.

INSERT FIGURE 3 HERE

Consider an A-chain located at site 0. Then, on average, there will be $6N_A/(N_A + N_B)$ A-chains as nearest neighbors around it and $6N_B/(N_A + N_B)$ B-chains. The van der Waals energy of the A-chain at site 0 will be approximately $-6AL_AN_A/(N_A + N_B) - 6AL_BN_B/(N_A + N_B)$. If there is a B-chain at site 0, then the van der Waals energy is approximately $-6AL_BN_A/(N_A + N_B) - 6AL_BN_B/(N_A + N_B)$ The total energy of this randomly interesterified crystal is then, approximately,

$$E_{tot\ interest} = N_A[-3AL_A N_A/(N_A+N_B) - 3AL_B N_B/(N_A+N_B)] + N_B[-3AL_B N_A/(N_A+N_B) - 3AL_B N_B/(N_A+N_B)]$$

$$= -3A[(L_A - L_B)N_A^2/(N_A+N_B) + L_B(N_A+N_B)] \qquad (5)$$

Now, we can write (4) as,

$$E_{tot\ pure} = -3A[(L_A - L_B)N_A + L_B(N_A+N_B)] \qquad (4')$$

If we assume, as is reasonable, that the energy of a liquid state oil is the same whether it is two separate TAG species or the interesterified TAGs, then, comparing (4') and (5), then, noting that the second terms in (4') and (5) are equal, the transition enthalpies differences will be approximately,

$$\Delta H_{tot\ pure} \sim N_A \qquad (6)$$

$$\Delta H_{tot\ interesterified} \sim N_A\, N_A/(N_A+N_B) \qquad (7)$$

We can then predict that the ratio of transition enthalpies will scale approximately like 1: $N_A/(N_A+N_B)$. Thus, if the ratio of B:A is 1:2 we expect that the transition enthalpy will be decreased from $\Delta H_{tot\ pure}$ to approximately $2\Delta H_{tot\ pure}/3$.

Experimental validation of this theory can be found in Table 4, where the decrease in the ratio of enthalpies for interesterified (IE) over non-interesterified (NIE) mixtures matches almost quantitatively the molar ratio of HOAO in the blend. Interestingly, the agreement was excellent for both crystallization as well as melting.

We have not considered the transition entropy which would enable us to predict the decrease in transition temperature from first principles. But we note that, while the entropy of the liquid state might be unchanged, the entropy of the solid state would be higher for the interesterified case, making the change in entropy upon melting smaller. This is mainly due to the increase in the number of molecular species in the system upon interesterification (Tables 1 and 2). However, the decrease in enthalpy upon interesterification is deemed to the dominant effect since the agreement between theory and experiment was excellent.

Conclusions

In this study we determined the effects of blending and interesterification on the physical properties of triacylglycerol oil blends. Although very small differences in the viscosity between chemically interesterified, enzymatically interesterified and non-interesterified oils were detected, the melting points and crystallization temperatures were strongly influenced by interesterification. TAG crystallization was highly dependent on TAG molecular structure, *i.e.*, by the fatty acid distribution within the TAG molecule. Although both viscosity and crystallization are dependent on the interactions between molecules, crystallization requires not only interaction, but also molecular structural complementarity to be able to pack into a crystal lattice. Introduction of shorter fatty acid chains into high oleic algal oil increased molecular heterogeneity, which was shown to decrease the enthalpy of melting drastically, leading to the observed decrease in melting temperature. Most vegetable oils have a viscosity and crystallization temperature significantly higher than mineral oil and synthetic lubricants, making their use problematic without modification. As a conclusion, in the absence of a suitable natural oil of low viscosity and high stability, incorporation of medium chain fatty acids into high oleic algal oil by chemical and enzymatic esterification constitutes a suitable method for not only decreasing the viscosity, but also engineering a significant drop in the crystallization and melting temperatures of vegetable oil-based lubricants.

Acknowledgements

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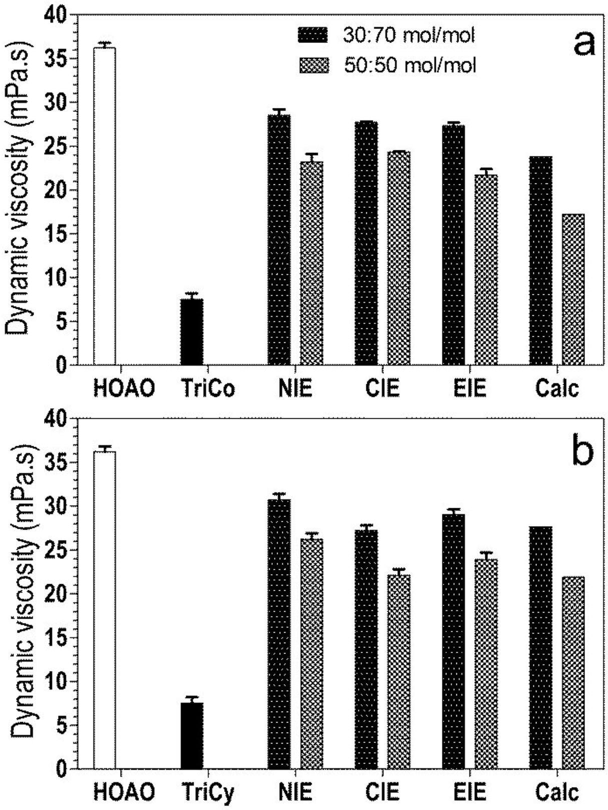

Figure 1 Dynamic viscosity of blends of tricaproin/HOAO (a) and tricaprylin/HOAO (b) at 40 °C. Abbreviations use are HOAO: high oleic algal oil, NIE: non-interesterified, CIE: chemically interesterified, EIE: enzymatically interesterified, TriCy: tricaprylin, TriCo: tricaproin, Calc: calculated. Values represent means and standard deviations of n=3 replicates.

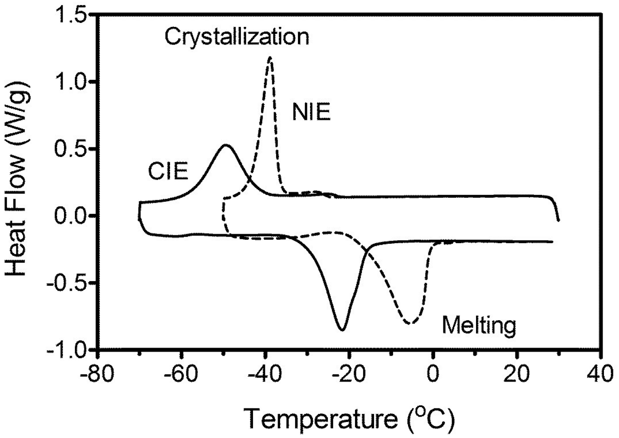

Figure 2 Differential Scanning Calorimetric trace of 50:50 (mol:mol) tricaprylin:high oleic algal oil mixtures before (NIE) and after chemical interesterification (CIE).

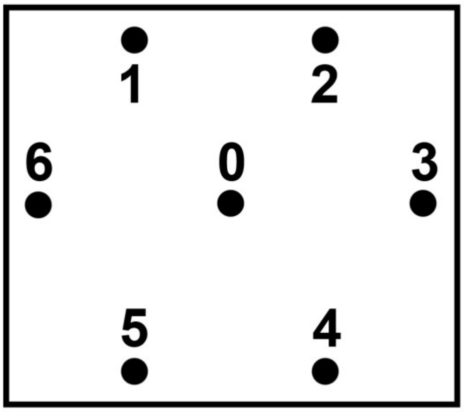

Figure 3 Labelled sites of a triangular lattice.

Table 1 Triacylglycerol composition of tricaproin-high oleic algal oil (HOAO) blends (mol:mol) which have not been interesterificed, and their chemically and enzymatically interesterified counterparts. Abbreviations used are Co: caproic acid, O: oleic acid, P: palmitic acid, S: stearic acid, Po: palmitoleic acid, M: myristic acid.

TAG	**Molecular Weight (g/mol)**	**Non interesterified**		**Chemically interesterified**		**Enzymatically interesterified**	
		50:50	**30:70**	**50:50**	**30:70**	**50:50**	**30:70**
CoCoCo	387.6	16.7	9.0	7.2	0.6	4.5	0.7
CoOCo	553.9	nd*	nd	8.4	5.1	23.0	8.3
CoOPo	692.1	nd	nd	nd	0.5	0.9	0.7
CoOM	666.1	nd	nd	nd	0.6	0.8	0.8
CoOO	720.2	nd	nd	16.2	23.6	34.1	30.3
CoOP	694.1	nd	nd	2.6	3.8	5.6	5.2
CoOS	722.2	nd	nd	nd	0.6	1.3	0.9
OOPo	858.4	1.2	1.7	0.6	1.2	0.3	0.8
OOM	832.4	1.6	1.7	1.1	1.5	0.4	1.2
OOO	886.5	57.6	63.9	37.4	42.3	14.7	30.6
OOP	860.4	16.4	18.3	11.0	10.9	4.1	8.0
POP	834.3	0.9	0.7	nd	0.5	nd	0.5
OOS	888.5	2.2	2.6	0.9	1.6	0.6	1.6

*nd; not detected

Table 2 Triacylglycerol composition of tricaprylin-high oleic algal oil (HOAO) blends (mol/mol) which have not been interesterified (NIE), and their chemically (CIE) and enzymatically (EIE) interesterified counterparts. Abbreviations used are Cy: caprylic acid, O: oleic acid, P: palmitic acid, S: stearic acid, Po: palmitoleic acid, M: myristic acid.

TAG	**Molecular Weight (g/mol)**	**NIE**		**Chemically interesterified**		**Enzymatically interesterified**	
		50:50	**30:70**	**50:50**	**30:70**	**50:50**	**30:70**
CyCyCy	471.6	19.2	10.4	5.8	1.1	6.7	1.0
CyOCy	609.9	nd*	nd	25.3	11.2	26.4	9.9
CyCyP	583.8	nd	nd	3.1	1.2	2.7	0.9
CyOPo	720.1	nd	nd	0.8	0.8	0.4	0.6
CyOM	694.1	nd	nd	1.0	1.0	0.6	0.7
CyOO	748.2	nd	nd	32.6	32.3	32.6	31.1
CyOP	722.1	nd	nd	5.3	5.9	5.5	5.4
CyOS	750.2	nd	nd	1.0	0.9	1.1	1.0
OOPo	858.4	1.5	1.8	nd	0.7	nd	1.1
OOM	832.4	1.9	2.3	nd	1.1	nd	1.3
OOO	886.5	54.6	60.4	13.0	29.3	12.4	30.2
OOP	860.4	16.5	17.9	3.1	8.0	2.7	7.2
POP	834.3	0.6	1.0	nd	0.4	nd	0.5
OOS	888.5	3.5	3.7	0.6	1.5	0.7	1.2

*nd: not detected

Table 3 Density, kinematic viscosity and average molecular weight of blends (mol:mol) of non-interesterified (NIE), chemically interesterified (CIE) and enzymatically interesterified (EIE) high oleic algal oil (HOAO) with tricaproin and tricaprylin at 40^{o} C.

	Density (g/cm^3)	**Kinematic Viscosity (cSt)***	**Average Molecular Weight (g/mol)**
HOAO	0.9137	39.6 ± 0.6^{a}	879.7
CIE HOAO	0.9134	36.8 ± 0.8^{a}	879.7
Tricaproin	0.9657	7.8 ± 0.7^{b}	386.5
Tricaprylin	0.9424	12.5 ± 0.7^{c}	470.7
Tricaproin/HOAO			
NIE 30:70	0.9252	30.8 ± 0.7^{a}	816.5
CIE 30:70	0.9211	30.1 ± 0.1^{a}	748.6
EIE 30:70	0.9250	29.5 ± 0.4^{a}	695.0
NIE 50:50	0.9346	24.8 ± 0.9^{b}	767.1
CIE 50:50	0.9305	26.1 ± 1.0^{b}	657.7
EIE 50:50	0.9340	23.2 ± 0.7^{c}	627.1
Tricaprylin/HOAO			
NIE 30:70	0.9203	33.3 ± 0.7^{a}	814.3
CIE 30:70	0.9206	30.5 ± 0.6^{b}	744.6
EIE 30:70	0.9225	31.4 ± 0.6^{b}	723.5
NIE 50:50	0.9259	28.2 ± 0.7^{c}	781.3
CIE 50:50	0.9272	26.3 ± 0.7^{d}	649.4
EIE 50:50	0.9295	25.7 ± 0.8^{d}	646.7

* Mean ± SD, n=3; Values with the same superscript are not significantly different (p>0.05) within each oil blend family

Table 4 Crystallization and melting temperatures and their corresponding enthalpies for of blends (mol:mol) of non-interesterified (NIE), chemically interesterified (CIE) and enzymatically interesterified (EIE) high oleic algal oil (HOAO) with tricaproin and tricaprylin*.

	Crystallization temperature (°C)	**Enthalpy of crystallization (ΔH_c), J/g**	**Melting temperature (°C)**	**Enthalpy of melting (ΔH_m), J/g**	**$\Delta H_{c,IE}/\Delta H_{c,NIE}$** Crystallization	**$\Delta H_{m,IE}/\Delta H_{m,NIE}$** Melting
HOAO	-34.8 ± 0.2^{a}	61.5 ± 0.2^{a}	-3.7 ± 0.2^{a}	88.79	-	-
CIE HOAO	-34.4 ± 0.2^{a}	61.3 ± 0.4^{a}	-5.6 ± 0.6^{a}	88.77	1	1
Tricaproin/HOAO						
NIE 30:70	-37.8 ± 1.5^{a}	52.8 ± 1.6^{a}	-6.1 ± 0.1^{a}	75.0 ± 0.8^{a}	-	-
CIE 30:70	-52.9 ± 0.0^{b}	36.2 ± 1.8^{b}	-16.1 ± 0.2^{b}	56.1 ± 5.9^{b}	0.69	0.70
EIE 30:70	-58.2 ± 0.4^{c}	33.6 ± 2.6^{b}	-19.7 ± 1.5^{c}	52.8 ± 0.6^{b}	0.69	0.69
NIE 50:50	-40.2 ± 0.1^{a}	44.4 ± 0.8^{c}	-8.5 ± 0.0^{a}	66.4 ± 2.4^{c}	-	-
CIE 50:50	-66.0 ± 1.0^{b}	20.3 ± 0.4^{d}	-23.3 ± 0.3^{b}	37.5 ± 1.9^{d}	0.47	0.51
EIE 50:50	-74.1 ± 0.3^{c}	17.4 ± 3.0^{d}	-28.2 ± 0.6^{c}	32.9 ± 0.05^{d}	0.46	0.49
Tricaprylin/HOAO						
NIE 30:70	-37.8 ± 1.3^{a}	58.5 ± 1.2^{a}	-4.5 ± 0.1^{a}	92.9 ± 12.6^{a}	-	-
CIE 30:70	-47.4 ± 1.0^{b}	$47.2 \pm 4.^{1b}$	-14.9 ± 1.2^{b}	69.0 ± 1.4^{b}	0.70	0.74
EIE 30:70	-58.3 ± 1.1^{c}	nd	-21.4 ± 0.8^{c}	nd	-	-
NIE 50:50	-39.5 ± 0.9^{a}	60.69 ± 0.02^{a}	-5.0 ± 0.0^{a}	82.7 ± 5.0^{a}	-	-
CIE 50:50	-47.9 ± 2.4^{b}	nd	-21.2 ± 0.7^{b}	Nd	-	-
EIE 50:50	-60.3 ± 0.5^{c}	nd	-21.1 ± 0.3^{c}	nd	-	-

* Values represent the mean ± SD, n=3. Values with the same superscript are not significantly different ($p>0.05$) within each oil blend family