Chemistry and Physics of Lipids 157 (2009) 113–119

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Characterization of the three major polymorphic forms and liquid state of tristearin by Raman spectroscopy

Eric Da Silva[a], Serge Bresson[b], Dérick Rousseau[a,*]

[a] Department of Chemistry and Biology, Ryerson University, 350 Victoria St., Toronto, Ontario, Canada, M5B 2K3
[b] Laboratoire de Physique des Systèmes Complexes, Université Picardie Jules Verne, 33 rue S[t] Leu 80039 Amiens Cedex, France

ARTICLE INFO

Article history:
Received 11 June 2008
Received in revised form 2 October 2008
Accepted 4 November 2008
Available online 13 November 2008

Keywords:
Raman spectroscopy
Triglyceride
Liquid state
Crystallization
Polymorphism
Enthalpy

ABSTRACT

Raman spectroscopy was used to distinguish the differences in the molecular organization of the α, β′ and β polymorphs, as well as the liquid state, of tristearin with focus placed on the C═O, C—H and C—C Raman-active stretching regions. The ester carbonyl stretching region permitted polymorphic discrimination due to significant differences in the number of modes, their relative frequencies and their full-widths at half-maximum. In the liquid state, the absence of obvious signatures in this region indicated that many local micro-environments likely exist about the ester carbonyl of molten tristearin. The ratio between the symmetrical and asymmetrical C—H stretching modes was linearly correlated with the enthalpy of fusion for each polymorph. The C—C stretching modes, which provided insight into the *trans*/*gauche* content, were polymorph independent, but changed significantly upon transition into the liquid state ($p < 0.05$). Overall, Raman spectroscopy allowed for the quick discrimination of tristearin polymorphs from a conformational and thermodynamic perspective.

1. Introduction

Solid-state triglycerides (TGs) exist in three key polymorphic forms identified by the lateral packing of their alkyl chains, namely the hexagonal (H) (α-form), orthorhombic ($O_{\perp}$) (β′-form) and triclinic ($T_{//}$) (β-form) subcells (Lutton, 1950; Malkin, 1954; Chapman, 1962; Lutton, 1972; Small, 1984; Larsson, 1994; Lutton, 1997). Different models for liquid state ordering in TGs have been proposed, including the smectic (Hernqvist and Larsson, 1982), nematic (Cebula et al., 1992) and discotic (Corkery et al., 2007) models, with computer simulation data pointing towards the latter being prevalent (Pink et al., 2008).

Fat crystallization involves the ordering of molten TG molecules into organized crystalline lattices. Upon cooling, liquid state TG molecules gradually organize into the H subcell, whose alkyl chains are packed loosely in comparison to the $O_{\perp}$ and $T_{//}$ subcells. Polymorphic transitions increase the stability of the lateral packing of the hydrocarbon chains. Many factors influence how a TG crystallizes from the melt, including composition, tempering regime, presence of other lipids or additives and mechanical treatment (shear, agitation, *etc.*). The polymorphic form that a TG crystal adopts plays a key role in the process engineering and consumer acceptability of many food products. For example, in tablespreads (butter, margarine, *etc.*), β′ crystals, which are typically needles <5 μm in length, are important for acceptable spreadability. In the manufacturing of chocolate, careful tempering is used to promote the crystallization of the metastable β-V form of cocoa butter, responsible for much of chocolate's organoleptic and shelf life properties. In this context, an understanding of TG molecular packing in the liquid and solid states can potentially aid in process optimization.

Many analytical techniques can be used to identify TG polymorphs, including X-ray diffraction (XRD), differential scanning calorimetry (DSC) and microscopy, though each of these techniques has its shortcomings. With XRD, structural elucidation is best achieved with single crystals, which can be difficult to isolate for most food-related TGs (Lutton, 1950; Malkin, 1954). With DSC strictly relying on thermal behavior, it is impossible to unambiguously confirm the polymorph present, based on melting point and enthalpy. Lastly, microscopy can provide information on TG crystal size and morphology, but no direct evidence of polymorphic form.

Two major vibrational spectroscopy-based techniques have been used to distinguish the molecular structure of TG polymorphs, namely infrared spectroscopy (Kobayashi et al., 1986; Kodali et al., 1989; Yano and Sato, 1999; Dohi et al., 2002; Chang et al., 2005; Yang et al., 2005) and Raman spectroscopy (Koyama and Ikeda, 1980; Simpson and Hagemann, 1982; Simpson, 1983; Hernqvist, 1984; Kobayashi et al., 1986; Kodali et al., 1989; Sprunt et al., 2000; Dohi et al., 2002; Yang et al., 2005; Meng et al., 2005;

* Corresponding author. Tel.: +1 416 979 5000x2155; fax: +1 416 979 5044.
E-mail address: rousseau@ryerson.ca (D. Rousseau).

0009-3084/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2008.11.002

Bresson et al., 2005, 2006; Da Silva and Rousseau, 2008). These techniques can also provide substantial structural and thermodynamic information (Da Silva and Rousseau, 2008). Early Raman spectroscopy-based studies led to the identification of key fingerprint regions for the methyl and methylene stretching as well as the C—C stretching of TGs (Larsson, 1966; Simpson, 1983; Larsson, 1994). More recently, Bresson et al. (2005, 2006) demonstrated that for five saturated monoacid triglycerides (n_C = 9, 11, 12, 14 and 16), the Raman active C=O stretching was a possible indicator of TG polymorph, although no XRD was used to corroborate these results.

This study presents the Raman spectra of the key polymorphic forms and liquid state of tristearin with emphasis placed on the evolution of the ester carbonyl stretching (1800–1700 cm^{-1}), along with complementary analysis and comparison of the Raman active C—C (1200–1000 cm^{-1}) and C—H (3000–2700 and 1500–1350 cm^{-1}) vibrational modes. Tristearin was chosen as a model TG as it has been well-characterized with XRD and DSC (Larsson, 1966; Lutton, 1972; Simpson and Hagemann, 1982; Aronhime et al., 1987; Hagemann, 1988; Elisabettini et al., 1996; Oh et al., 2002; MacNaughtan et al., 2006) and its polymorphs are easy to prepare.

2. Materials and methods

2.1. Preparation of tristearin polymorphs

Tristearin (purity ≥99%, Fluka Scientific, Oakville, Ontario) was used without further purification. The three polymorphic forms of tristearin were prepared using a DSC (Pyris Diamond, PerkinElmer, Markham, Ontario, Canada) with a precision of ±0.01 °C. Calibration was performed in the higher temperature range with an indium standard and at the lower temperature range using a water standard obtained from a Milli-Q® water purification system (18.2 MΩ cm).

Samples (7–8 mg) of molten tristearin (brought to ~90 °C) were placed in aluminum DSC pans and sealed. All samples where initially brought to 90.00 °C at a rate of 50.00 °C min^{-1} and held for 5 min to remove any crystalline history/memory.

For the preparation of the α polymorph, the tristearin sample was cooled from 90.00 °C to 25.00 °C at 50.00 °C min^{-1} and allowed to crystallize for 10 min. For the preparation of the β′ polymorph, tristearin was brought to 57.00 °C at 50.00 °C min^{-1} from the 90.00 °C melt (MacNaughtan et al., 2006) and held isothermally for 100 min, which was the minimum time necessary for full crystallization (Fig. 1). Though two sub-forms of the β′ polymorph have been identified, namely the β_1' and β_2' forms (Simpson, 1983), no attempts were made to isolate them. The β polymorph was prepared by cooling tristearin at 50.00 °C min^{-1} to 61.00 °C from the 90.00 °C melt and held isothermally for 60 min (MacNaughtan et al., 2006) (Fig. 1). All samples were cooled to 25.00 °C prior to Raman analysis.

2.2. Raman spectroscopy

Raman spectroscopy was performed using a Renishaw confocal Raman microscope (Renishaw Ramascope 2000, Gloucestershire, UK) and analysis was performed with the accompanying Renishaw Wire® software. The Raman microscope was equipped with an energy dispersive CCD detector with a resolution of better than 2.5 cm^{-1}. Excitation of the sample was achieved using a 782 nm near IR diode laser. Spectra were collected using a 180° backscatter geometry to the sample with a 20× objective lens and a laser power of 2.00 ± 0.09 mW, measured at the sample using a laser power probe with an accuracy of ±5% (Coherent LaserCheck, Coherent Inc., Auburn, CA, USA). No changes in sample temperature greater than ±0.01 °C were observed. Samples were prepared for Raman spectroscopy by removal of the DSC pan lids, with analysis performed *in situ*. No interference was observed from the aluminum pans as sample weights were sufficient to cover the DSC pan aluminum surface. Spectral scans between 3500 cm^{-1} and 500 cm^{-1} were performed using a 150 s scan time. The region between 1800 cm^{-1} and 1700 cm^{-1} was further scanned with 5 accumulations of 150 s each to permit spectral curve fitting in the region by increasing the signal-to-noise ratio (background signal reduction by a factor of $\sim 5^{1/2}$). Acquired spectra were normalized to the 1297 cm^{-1} Raman line prior to analysis. All spectra were acquired with the cosmic ray removal function on. Curve fitting of the ester carbonyl region was performed as per Da Silva and Rousseau (2008).

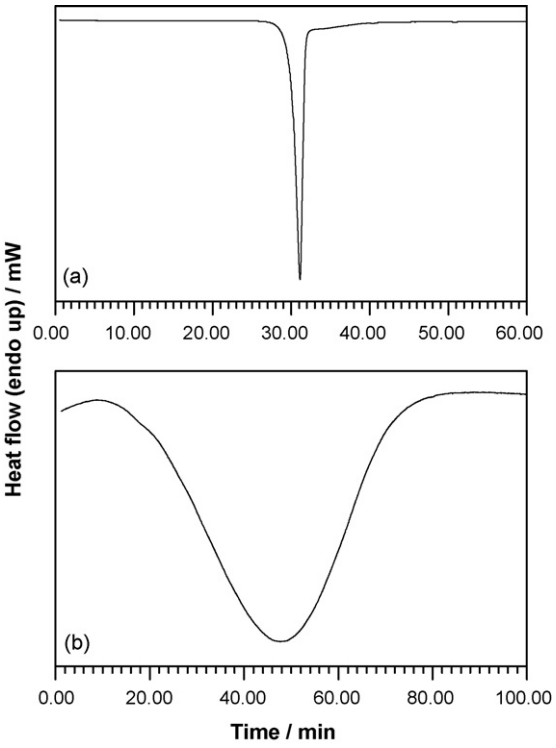

Fig. 1. Isothermal crystallization of tristearin at (a) 61.00 °C (β polymorph) and (b) 57.00 °C (β′ polymorph).

2.3. Polymorphic discrimination

The melting behavior and enthalpies of fusion determined with DSC were used to confirm polymorph identity at the time of study. Samples were heated from 25.00 °C to 90.00 °C at 40.00 °C min^{-1} to avoid any polymorphic transitions during melting (Fig. 2). Confirmation was performed on samples immediately after polymorph preparation and after Raman analysis. Further confirmation was obtained with powder X-ray diffraction (XRD) (Rigaku Geigerflex, Danvers, MA, USA). The XRD diffractograms were collected using a Co source with a K_{avg} of 1.79021 Å ($K_{\alpha 1}$ = 1.78892 Å; $K_{\alpha 2}$ = 1.79278 Å) using an anode current of 40 mA, an anode voltage of 50 kV and a scan speed of 2° min^{-1}. XRD diffractograms were collected in the 1.55–35.00° (2θ) (1.56–33.09 Å) range at room temperature and within 15 min of polymorph synthesis. Samples were prepared using temperature regimes close to those used for the DSC methodology, but using ovens and glass XRD sample holders.