Crystal Structure of Hydrogenated Canola Oil

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ABSTRACT

Scanning electron microscopy was used to study the crystal structure of hydrogenated Canola oil. Fixation of the samples was carried out at 10 C using osmium tetroxide vapor in a sealed container. Tristearin was used as a standard and was prepared in its three reported polymorphic forms α, β' and β. Crystallization from ethyl acetate gave either a mixture of β' and β or pure β' polymorphic forms depending on the concentration of tristearin. Micrographs of these forms were obtained showing the morphology of the crystals. A Canola sample which was selectively hydrogenated to an iodine value of 70 was detergent fractionated, washed, dried and subjected to the same fixation procedure. Micrographs were obtained of its predominant β-polymorphic form.

INTRODUCTION

Microscopy has been used extensively to study the microstructure of fats and fat products. Work done in this area has been thoroughly reviewed (1,2). Electron microscopy investigations carried out on fats and fat products essentially use biological preparation techniques adapted to each sample studied. These techniques involve either thin sectioning followed by fixation in aqueous osmium tetroxide at room temperature (3), or replica techniques sometimes carried out under controlled temperature conditions (4,5,6). The study of the microstructure of fat crystals is a difficult task due to the following factors:

- fat crystals are highly influenced by temperature variation.
- fat crystals undergo polymorphic transitions resulting in crystal lattice rearrangement and possible morphology changes.

These factors must be considered and hence special techniques are required. Due to the instability of fat crystals and possible uncontrolled reactions occurring between osmium tetroxide and triglyceride molecules (7,8), little work has been done in this area. The present work describes a modification of the fixation step, and involves the use of osmium tetroxide vapor at controlled temperatures rather than the traditional fixation with aqueous osmium tetroxide.

MATERIALS AND METHODS

Commercially refined and bleached Canola oil used in this study was obtained from CSP Food Ltd., Man. Hydrogenation was carried out under selective conditions (200 C; 48 kPa hydrogen pressure) to an iodine value of 70 in a Parr pressure hydrogenator. The hydrogenated oil was further treated with dioctylsulfosuccinate according to the method described by Poot et al. (9) to remove most of the liquid oil. Tristearin was obtained from ICN Pharmaceuticals Inc., Cleveland, Ohio (m.p. 65-67 C) and further recrystallized from acetone. Its purity was checked on GLC (DEGS 12%). The three polymorphic forms of tristearin were obtained as follows:

- tristearin crystallized in its β-modification from acetone (m.p. 73 C).
- the β'-modification (m.p. 64 C) was obtained by crystallization from the melt a few degrees above the α-form melting point, or by crystallization from ethyl acetate.
- the α-modification was obtained by rapid cooling of the melt.
- a mixture of β' + β-forms also was obtained by crystallization from ethyl acetate.

X-ray diffraction patterns of all the samples were run at 10 C prior to fixation using a Guinier camera model FR 552 of Enraf-Nonius, Delft, The Netherlands. Infra-red spectra were run using a Beckman model 4320 instrument. KBr discs of the samples before and after fixation were prepared to determine possible changes occurring during fixation. C^{13} NMR spectra were run using a Bruker CXP/100 with a home built solid state CP-MAS probe. Osmium tetroxide was 99.95% pure, purchased in 0.5 g ampules (Can-em Chemical Distributors, Guelph, Ont.). The samples were placed on aluminum stubs and fixed in a closed container at 10 C for 24 hr. They were then coated with gold/palladium 30-40 nm in a Hummer V sputter coater. An ETEC autoscan scanning electron microscope was used to examine the samples at an accelerating voltage of 5 kv. A polarizing light microscope Model BH with a PM-6 Olympus camera attachment was used to view the fractionated Canola sample.

RESULTS AND DISCUSSION

An examination of the micrographs obtained for the three polymorphic forms of tristearin shows the following features after osmium tetroxide fixation: the appearance of

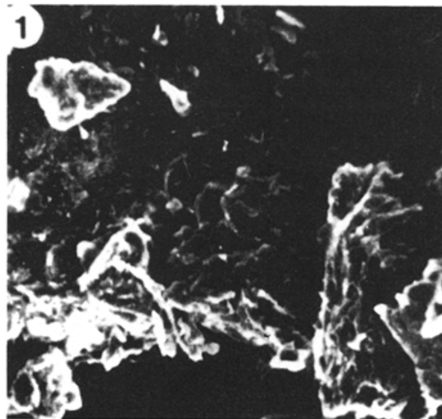

FIG. 1. Micrograph of the α-polymorphic form of tristearin after fixation with OsO_4, as viewed by the scanning electron microscope (magnification 800 times).

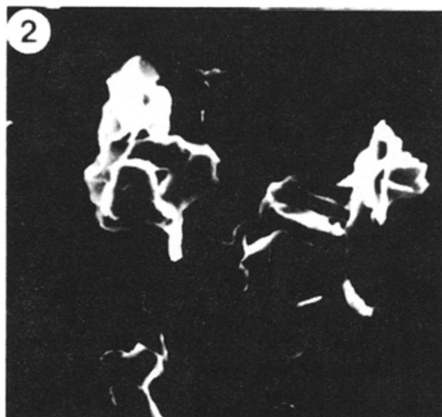

FIG. 2. Micrograph of the β'-polymorphic form of tristearin after fixation with OsO_4, as viewed by the scanning electron microscope (magnification 800 times).

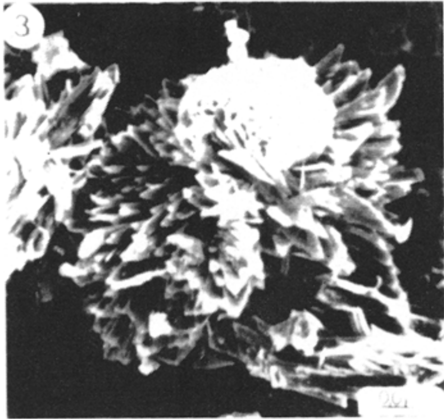

FIG. 3. Micrograph of the β-polymorphic form of tristearin after fixation with OsO_4, as viewed by the scanning electron microscope (magnification 800 times).

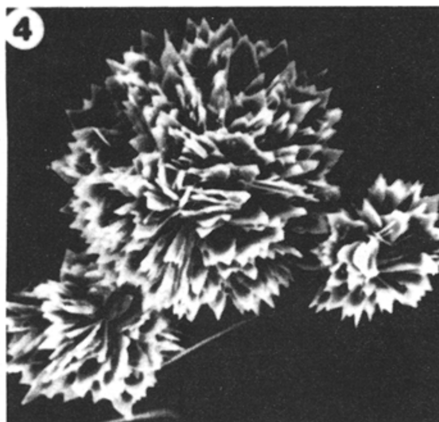

FIG. 4. Micrograph of the β-polymorphic form of fractionated Canola crystals after fixation with OsO_4, as viewed by the scanning electron microscope (magnification 800 times).

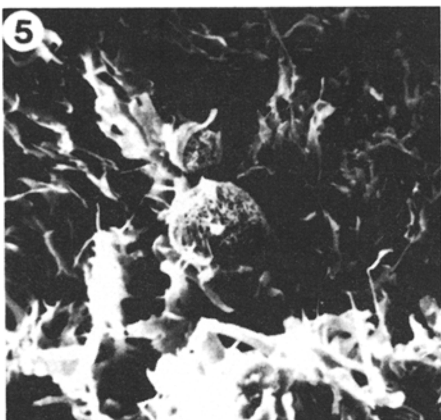

FIG. 5. Micrograph of β′ + β-polymorphic forms of tristearin (crystallized from ethyl acetate), as viewed by the scanning electron microscope (magnification 800 times).

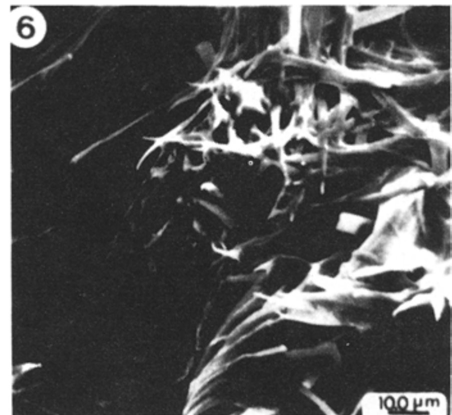

FIG. 6. Micrograph of β′-polymorphic form of tristearin (crystallized from ethyl acetate), as viewed by the scanning electron microscope (magnification 800 times).

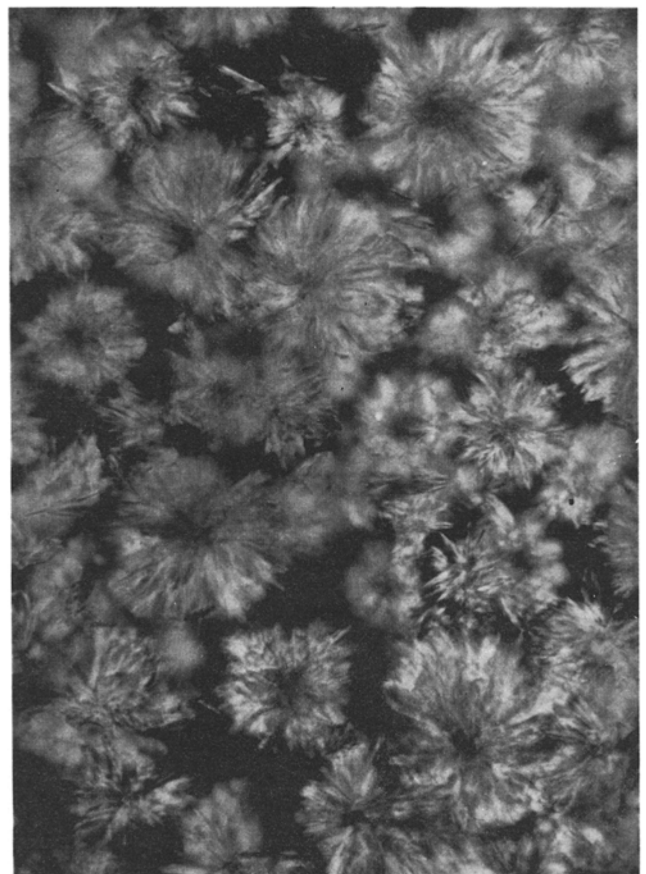

FIG. 7. Micrograph of the β-polymorphic form of fractionated Canola crystals, as viewed by the polarized light microscope (magnification 400 times).

the surface of the α-polymorphic form (Fig. 1) is relatively smooth with no defined structure; the β′-polymorphic form (Fig. 2) shows crystals in the form of plates packed loosely together and varying in size from 5-10 μm; the β-polymorphic form (Fig. 3) consists of tightly packed spherulites with pointed edges that varied in size between 20-30 μm.

Fractionated Canola was obtained in the β-form, and the micrograph of the crystals after fixation (Fig. 4) consisted of spherulites identical to the ones observed for tristearin in the β-form. Polarized light microscopy of the same sample (Fig. 7) also showed the crystals as clusters of well defined spherulites.

When tristearin was crystallized from ethyl acetate, the polymorphic forms depended on concentration. X-ray diffraction patterns of the forms present at the four different concentrations studied are presented in Table I. A mixture of β′ + β forms is evident at the lowest and highest concentrations. The β′-form is predominant at the intermediate concentrations. Micrographs of the former samples after osmium tetroxide fixation (Fig. 5) clearly show the β′-form consisting of a network of needle-shaped crystals surrounding the well defined spherulites representing the β-form. Micrographs of the latter samples show tristearin crystals predominantly in the β′-form (Fig. 6).

TABLE I

X-ray Diffraction Patterns of Tristearin in Ethyl Acetate

Sample number	Sample conc. (wt %)	Short spacings (A)				Chain packing
1	11.3	4.6	4.2	3.8	3.7	β' + β
2	5.9	–	4.2	3.8	–	β
3	2.7	–	4.2	3.8	–	β
4	0.5	4.6	4.2	3.8	3.7	β' + β

Spectroscopic evidence so far obtained indicates that the molecular structure of the triglyceride molecules remained unchanged during the course of fixation. Infra-red spectra of tristearin, before and after fixation were identical. The infra-red spectrum of fractionated Canola exhibited an additional band near 980 cm^{-1} after fixation which can be due to the formation of monoester as a product of the reaction of a double bond with osmium tetroxide. Reaction of the double bonds in unsaturated fats with osmium tetroxide has been postulated by other researchers (10) and various structures have been proposed; the most generally accepted involves four coordinate osmium VI. The formation of such a structure does not, however, change the morphology of the crystals which is evident from the identical appearance of the spherulites in the micrographs showing the β-form of tristearin (Fig. 3) and that of fractionated Canola (Fig. 4). C^{13} spectra obtained for the β-form of tristearin after fixation also confirmed that the molecular structure remained essentially intact.

This simple and fast method for crystal fixation provides a technique that is very promising for studying fats and fat products using the high resolution capabilities of scanning electron microscopy.

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✻Lipid Methodology – Chromatography and Beyond. Part II. GC/MS, LC/MS and Specific Enzymic Hydrolysis of Glycerolipids

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ABSTRACT

The combination of specific enzymic degradation with GC/MS or LC/MS identification and quantitation of enantiomeric diacylglycerols and reverse isomers has greatly improved the methods of structural analysis of triacylglycerols, so that in many instances complete characterization of both major and minor species is possible. The techniques described for the analysis of triacylglycerols and sn-1,2- and sn-2,3-diacylglycerols are also applicable to the X-1,3-diacylglycerols and X-1-monoacylglycerols following coversion to triacylglycerols by acylation with appropriate fatty acids. For many applications, however, a combination of specific enzymic hydrolysis with a GLC analysis of the products on polar capillary columns may be adequate for the identification and quantitation of the major molecular species of both triacylglycerols and diacylglycerols.

INTRODUCTION

In Part I of this series (1) we demonstrated that a combination of either GLC or HPLC with mass spectrometry was necessary for an unequivocal identification of the chemical composition and molecular association of the fatty acids in the resolved triacylglycerol and diacylglycerol species. Except for certain reverse isomers of diacylglycerols, these techniques do not allow the identification of positional isomers or enantiomers of acylglycerols. In the past, positional distribution of fatty acids in triacylglycerols has been determined by the stereospecific analysis of Brockerhoff (2), but this method gives no information about the molecular association of the fatty acids, unless the triacylglycerol mixture first has been resolved into individual molecular species, which is impractical. Likewise, no indication of molecular association is obtained by the positional analysis of fatty acids in the glycerophospholipids commonly performed by phospholipase A_2 (3). Myher and Kuksis (4) have proposed a method of stereospecific analysis which overcomes this difficulty. In their routine the X-1,2-diacylglycerols are converted into the corresponding X-1,2-diacylphosphatidylcholines, which are then subjected to a stepwise stereospecific release of the sn-1,2- and the sn-2,3-diacylglycerol moieties by digestion with phospholipase C. The enantiomeric diacylglycerols then can be submitted to a complete chromatographic resolution. The problem of a physical separation of the reverse isomers remains, but their proportion can be determined by collecting enantiomerically pure molecular species of diacylglycerols by HPLC and subjecting them to hydrolysis with pancreatic lipase in the form of the tertiary-butyldimethylsilyl (t-BDMS) ethers.

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