Chemistry and Physics of Lipids 198 (2016) 1–9

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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

Microscopic approach of the crystallization of tripalmitin and tristearin by microscopy

Roberta Claro da Silva*, Fabiana Andrea Schafer De Martini Soares, Jéssica Mayumi Maruyama, Natália Roque Dagostinho, Ylana Adami Silva, Juliana Neves Rodrigues Ract, Luiz Antonio Gioielli

Department of Biochemical and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes n. 580, B16, CEP 05508-900, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 4 April 2016
Accepted 11 April 2016
Available online 14 April 2016

Keywords:
Triacylglycerols
Microscopy
Tripalmitin
Tristearin
Polymorphism

ABSTRACT

The crystallization behavior of lipids has important implications in industrial processing of food products, whose physical characteristics depend largely on crystallized fats. The study of the crystallization behavior and polymorphism of a pure lipid system is of great scientific importance as a means of gaining an understanding of the phenomena involved, serving as basic knowledge to help guide the addition or removal of these compounds in different raw materials. The crystallization behavior and polymorphism of pure tripalmitin (PPP) and tristearin (SSS) were investigated by Polarized Light Microscopy (PLM) and Differential Scanning Calorimetry (DSC) under different crystallization conditions. The polymorphic forms (β′ and β) of PPP and SSS exhibited different morphologies depending on how they were obtained, either from α form recrystallization or from isotropic melt. Crystallization in the β form was faster in SSS than in PPP, indicating that the process occurs faster in TAGs composed of longer fatty acid chains. Both β′ and β polymorphic forms were obtained from α form recrystallization, albeit with predominance of the β form.

1. Introduction

Triacylglycerols (TAGs) are major constituents of fats and oils and biologically important organic molecules along with proteins and carbohydrates. In industrial applications, TAGs represent the main components in cream, margarine and confectionery fats in foods, and act as matrix material in pharmaceuticals and cosmetics. The physical behavior of TAGs influences the physical properties of fat-based products, such as appearance, texture, plasticity, morphology, and rheology. Most fat-based products are multicomponent TAG mixtures, containing different kinds of fatty acid moieties (Sato and Ueno, 2005).

Tripalmitin (PPP) is used for the synthesis of human milk fat substitute (HMFS), the richest energy source in infant formulas. HMFS can be synthesized from PPP and vegetable oils rich in oleic acid by interesterification using an *sn*-1,3 specific lipase as a biocatalyst (Maduko et al., 2007). The fraction rich in PPP can be separated from palm stearin by acetone fractionation (Mae Son et al., 2010).

Tristearin (SSS) is the main triacylglycerol in fully hydrogenated soybean oil (FHSBO), a relatively low-cost product obtained by total hydrogenation that can be used as hardstock for producing interesterified *trans*-free fat bases applied in shortenings and bakery margarines (Ribeiro et al., 2009a; Maleky et al., 2012). FHSBO contains over 63% SSS (Ribeiro et al., 2009b; Garcia-Macias et al., 2012) and these levels are expected to increase after fractionation processes.

In many food products, and also in some processing operations, strict control of lipid crystallization is indispensable to achieve the desired number, size distribution, polymorphic form, and dispersion of the crystalline phase. In most foods, crystallization of TAGs is the most important attribute influencing product quality, although crystallization of other lipids (i.e., monoacylglycerols, diacylglycerols, phospholipids, and others) may also be important. Size distribution (mean size and range of sizes), polymorphic form, and shape of the fat crystals, as well as the resulting network formed, all play important roles in determining physical attributes of lipid-based products (Metin and Hartel, 2005; Rousseau et al., 1998).

* Corresponding author.
E-mail addresses: roberta@usp.br, clarorob@yahoo.com.br (R.C.d. Silva).

http://dx.doi.org/10.1016/j.chemphyslip.2016.04.004
0009-3084/

Various methodologies for studying the polymorphism of fats, including differential scanning calorimetry (DSC), X-ray diffraction (XRD), infrared absorption spectroscopy, and nuclear magnetic resonance (NMR), have been widely used (Campos, 2012). DSC analysis provides temperatures and enthalpy values of melting, crystallization, and polymorphic transition, which are prerequisites for isolation of individual polymorphic forms and determination of their thermal stability (Sato and Ueno, 2005). Microscopy allows the observation of dynamic changes that occur during nucleation and crystal growth (Maleky et al., 2012; Gamboa and Gioielli, 2006; Narine and Marangoni, 1999). Polarized light microscopy (PLM) is the most widely used technique for visualizing the microstructural network of fats and has been applied with the objective of explaining differences between the texture of fat blends, disclosing diverse types of crystals and morphological changes in crystal growth (Ribeiro et al., 2009b). Under certain circumstances, PLM can even distinguish the polymorphic forms of fats based on crystal form and size, aside from verifying transformations in the polymorphic forms (Ribeiro et al., 2009c; Kerr et al., 2011; Ray et al., 2012; Bouzidi and Narine, 2012). However, few studies have investigated the polymorphism of pure TAG systems using the PLM technique. Kellens et al. (1992) presented a detailed description of PPP morphology using PLM. Oh et al. (2002) induced polymorphic forms from SSS melts and characterized their crystalline morphology.

The study of the crystallization behavior and polymorphism of a pure lipid system is of great scientific importance as a means of gaining an understanding of the phenomena involved, serving as basic knowledge to help guide the addition or removal of these compounds in different raw materials. In the present study, pure tripalmitin and tristearin were submitted to different crystallization temperature conditions to determine their crystallization behavior and polymorphic transitions by Polarized Light Microscopy and Differential Scanning Calorimetry.

2. Materials and methods

2.1. Materials

Two pure monoacid triacylglycerols (tripalmitin and tristearin) were purchased from Sigma-Aldrich (UK), both of which had a purity higher than 99%.

2.2. Methods

2.2.1. Differential scanning calorimetry

Thermal analysis was performed on a DSC 4000 calorimeter (Perkin-Elmer USA). Lipid samples weighing approximately 5 mg were placed into 50 μL aluminum pans (BO14-3017 container, Perkin Elmer, USA) and hermetically sealed (lid BO14-3003, Perkin-Elmer, USA). The samples were first heated to 100 °C and held for 10 min to obtain an isotropic melt. Thereafter, the samples were cooled at a rate of 30 K/min to 50.5 °C for tripalmitin and 60.5 °C for tristearin. Samples were then held at the isothermal holding temperature for 150 min. During this time, crystallization was monitored by observing the isothermal heat flow. The samples were then heated at a rate of 5 K/min up to 100 °C to provide a melting endotherm for polymorph identification. Samples were

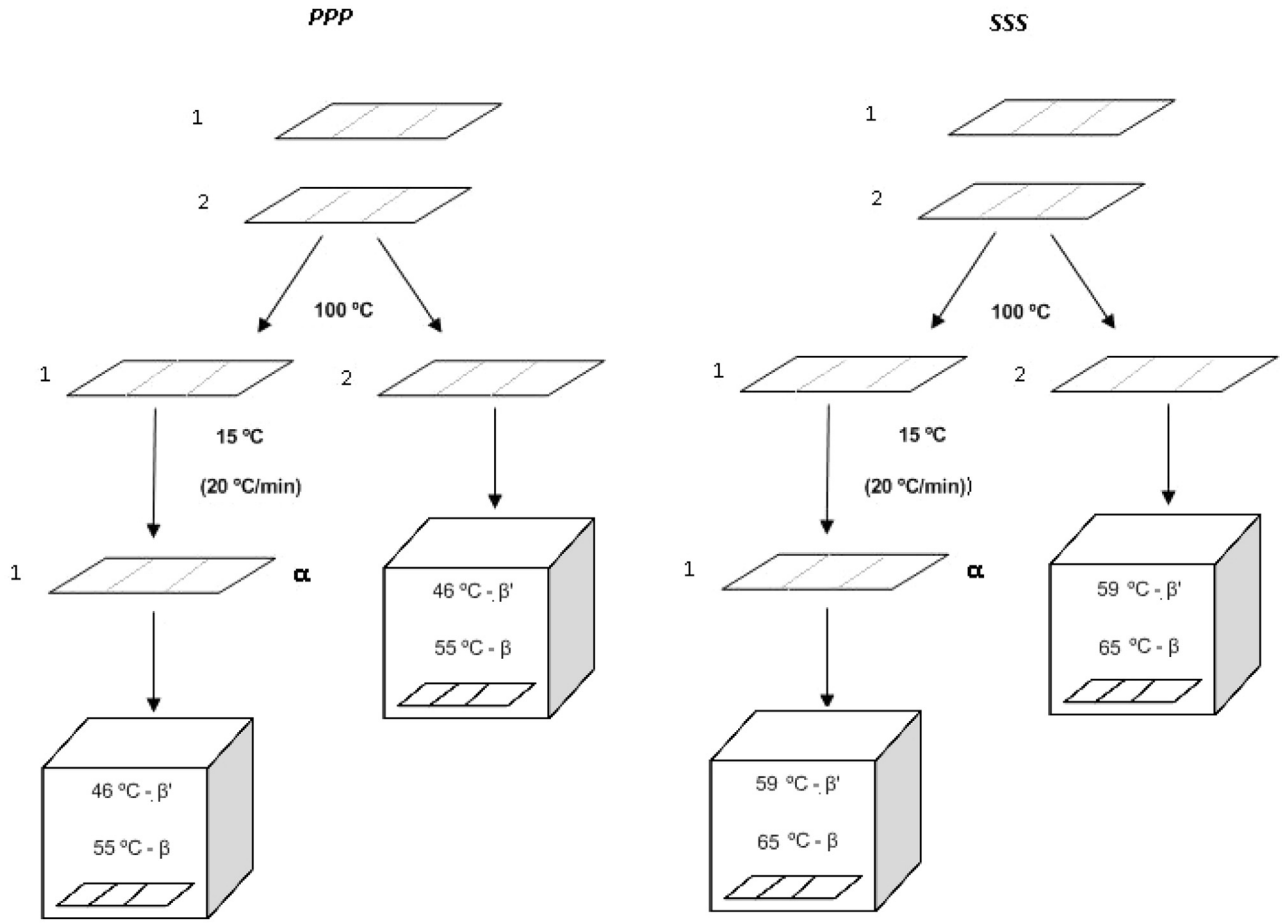

Fig. 1. Scheme of protocol to obtain polymorphic forms of PPP and SSS for polarized light microscopy.