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Unsaturated fatty acid enriched vs. control milk triacylglycerols: Solid and liquid TAG phases examined by synchrotron radiation X-ray diffraction coupled with DSC

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ABSTRACT

The fatty acid composition of milk triacylglycerols (TAGs) can be modified to improve their nutritional properties and the long-term health of consumers. However, the consequences of an increase in unsaturated fatty acid (UFA) content on the physical properties of milk TAGs remain to be elucidated. This study aimed at comparing the crystallisation properties and melting behaviour of control vs. UFA-enriched milk TAGs using the coupling of time-resolved synchrotron X-ray diffraction and differential scanning calorimetry on cooling and then heating at 3 °C/min. On cooling from the melt, high crystallisation temperature (HCT) TAGs solidify from 15.8 °C in α 2L (45–49 Å) structures, then low crystallisation temperature (LCT) TAGs form α 3L structures with a higher thickness (75.5 Å vs. 71.5 Å) and a delay (8.5 °C vs. 12.1 °C) for UFA-enriched TAG. On heating, melting of TAGs crystals and formation of $3L_2$ (64.7–80.1 Å) and β′ $2L_f$ (40–44 Å) crystals associated with polymorphic reorganisations have been characterised. The organisation of TAG molecules in their liquid state revealed a higher thickness for UFA-enriched TAGs as compared to control TAGs. Such results improve the knowledge about the mechanisms of TAG crystallisation as a function of their FA composition and are important for the technological utilisation of UFA-enriched TAGs in food products.

1. Introduction

Crystallisation of triacylglycerols (TAGs; triesters of fatty acids with glycerol) and polymorphism are important for technical fat processing and applicability in the food, pharmaceutical and cosmetic industries (Hagemann, 1988; Marangoni, Acevedo, Maleky, & Co, 2012; Sato, Bayes-Garcia, Calvet, Cuevas-Diarte, & Ueno, 2013). The texture, mouth-feel and rheological properties of high-fat content food products (e.g. butter, creams, spreads, chocolate) are greatly affected by the chemical composition and the physical properties of TAGs, which are the main components of dietary fats. The solid fat phase (i.e. crystals) formed at refrigerated and/or room temperature is due to the presence of saturated fatty acids (SFAs) within TAG molecules.

Among food products, milk and dairy products have received a particular attention. The composition of milk fat is complex: more than 400 fatty acids (FAs) characterised by various chain lengths (i.e. from 4 up to 24 atoms of carbon) and degrees of unsaturation have been identified (Jensen & Newburg, 1995). Milk fat contains 65 to 70% SFAs, mainly C16:0, C18:0 and C14:0 (Jensen & Newburg, 1995). Therefore, milk fat is a multi-component mixture containing a combination of more than 200 TAG species (Gresti, Bugaut, Maniongui, & Bezard, 1993). This complex composition of milk TAGs leads to specific physical properties. Milk TAGs are partially crystallised, i.e. a mixture of solid TAGs and liquid oil, over a wide range of temperatures spanning from −40 °C to 40 °C (Lopez, Bourgaux, Lesieur, Riaublanc, & Ollivon, 2006; Timms, 1980). The physical behaviour of milk TAG crystals is a determining factor in controlling the microstructure of many food products, such as butter, whipped cream, ice-cream and bakery applications. The mechanisms of milk TAG crystallisation are also involved in the fractionation of milk fat (Kaylegian & Lindsay, 1994; Lopez et al., 2006).

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For the last decades, decreasing the human consumption of SFAs in favour of unsaturated fatty acids (UFAs) has been recommended by health authorities to prevent cardiovascular disease risks and obesity (Joint FAO/WHO Expert Consultation, 2003). The chemical composition of milk fat in dairy products could be adapted as regards the nutritional recommendations. Hence, changing the FA composition of milk TAGs (98% of milk lipids; Christie, 1995) to contribute in the improvement of the long-term health of the consumers is of considerable interest for the dairy industry. However, decreasing the SFA content of milk TAGs and then increasing the UFA content in milk TAGs for nutritional and health reasons could have consequences on the physical properties of dairy products (melting, rheology, texture), that remain poorly known. In particular, the crystallisation properties and phase behaviour as a function of temperature could be affected. The presence of at least one double bond in a hydrocarbon chain causes rigid kinks in the UFAs and affects the packing of TAG structures compared to SFAs (Himawan, Starov, & Stapley, 2006). As a result, TAGs containing double bonds have lower melting points than saturated TAGs. Then, increasing the UFA content of milk TAGs will undoubtedly alter their physical properties and the texture of food products and could have consequences on the consumer acceptability. As regards this nutritional and health context and the potential consequences on products, UFA-enriched TAGs are attracting considerable attention in various fields of science and food technology. Although the understanding of the physical behaviour of UFA-enriched milk TAGs is of primary importance, information remains poor (Bugeat et al., 2011; Smet et al., 2010) and needs to be improved.

The objective of this work was to perform a comparative analysis of the crystallisation properties of control vs. UFA-enriched milk TAGs and their melting behaviour with examination of phase transitions occurring on heating. Also, this study aimed at comparing the organisation of the liquid phase as a function of the fatty acid composition of milk TAGs. Experiments have been conducted using high-sensitivity DSC and time-resolved synchrotron XRD recorded at both small and wide angles on cooling and subsequent heating at the rate of $|dT/dt| = 3$ °C/min. Synchrotron radiation XRD has the best facility to assess the rapid crystallisation, melting and reorganisations of TAG molecules, providing highly accurate structure information.

2. Materials and methods

2.1. Control and UFA-enriched milk TAGs

Control and UFA-enriched milk TAGs were naturally produced by cows fed a standard diet or a diet enriched in UFAs, respectively. The milks were obtained from two groups of 6 cows each with similar weight, lactation stage and milk production. The experimental diets were offered during 4 weeks. The cows received i) 1.7 kg dry matter of hay and grass silage ad libitum and ii) either 3.1 kg dry matter of cereals (control TAG group) or a diet rich in poly-UFAs composed of 0.6 kg dry matter of soybean meal (70% barley and 30% maize) and 0.7 kg of linseed oil (UFA-enriched TAG group). Linseed oil was dispersed on the silage and the quantities of oil were adjusted individually to bring an oil level of 5% dry matter for each cow. The milk from evening and morning milkings of each group of cows was mixed. Milk fat globules containing the TAGs were concentrated at 45 °C by centrifugation with a plate-separator (Elecrem, Vanves, France) to about 50% v/v fat content. The TAGs were extracted by churning the concentrated creams at 10 °C, heating at 70 °C for 10 min, and centrifugation at 1500 rpm at 55 °C for 10 min to recover the upper phase containing the control TAGs and UFA-enriched TAG. TAGs samples were stored at −20 °C until further experiments.

2.2. Fatty acid composition

The fatty acid composition of the milk fats has been determined by a method adapted from Park and Goins (1994) and reported in Bugeat et al. (2011). Methyl esters obtained from fatty acids of total lipids were analysed by gas chromatography (Agilent 7890, Santa Clara, California) with instrument parameters similar as the one used by Bugeat et al. (2011). The analyses were performed in triplicate.

2.3. Coupling of differential scanning calorimetry (DSC) and time-resolved synchrotron X-ray diffraction (XRD)

The coupled DSC and X-ray scattering experiments were performed on the SWING beamline at synchrotron SOLEIL (Gif-sur-Yvette, France). The set-up (Fig. 1) consisted in the insertion of a laboratory-built microcalorimeter designed to allow simultaneous thermal and temperature-controlled X-ray measurements from the same sample (Microcalix®, Ollivon et al., 2006) on the SWING X-ray beamline operating at 15 keV. A two-dimensional detector with sample to detector distance of 521 mm allowed the recording of XRD patterns in the range 0.08 $Å^{-1}$ to 1.8 $Å^{-1}$, thus covering both the small and wide-angle regions of interest. XRD at small angles allows the identification of the longitudinal stacking of TAG molecules in the lamellar structures while XRD at wide angles provides information on the lateral packing of their FA chains (subcell structure; α, β′, β). Intensity values were normalized to account for beam intensity, acquisition time and sample transmission. Each powder-like diffraction pattern, displaying a series of concentric rings, was then integrated circularly to yield the intensity as a function of the radial scattering vector q. The scattered intensity was reported as a function of the scattering vector $q = 4\pi\sin\theta / \lambda$, where 2θ is the scattering angle and λ is the wavelength of the incident beam. The channel to scattering vector q calibration of the detector was carried out with pure tristearin (β 2L form) (Lavigne, Bourgaux, & Ollivon, 1993) and silver behenate (Blanton, Barnes, & Lelental, 2000).

Small volumes (around 20 μL) of TAG samples preheated at 60 °C were loaded in thin quartz capillaries of 1.5 mm diameter (GLAS W. Muller, Berlin, Germany) and inserted in the Microcalix calorimeter cell pre-heated to 60 °C. The samples were submitted to a controlled cooling at a rate of $|dT/dt| = 3$ °C/min from 60 °C up to −5 °C while both DSC signals and XRD patterns were simultaneously recorded. The temperature of −5 °C has been chosen to be able to compare the crystallisation behaviour of TAG in bulk (this study) and dispersed within emulsion droplets (next paper in preparation), avoiding the formation of ice crystals. The samples were then submitted to a controlled heating at a rate of $dT/dt = 3$ °C/min from −5 °C up to 60 °C while both DSC signals and XRD patterns were simultaneously recorded. During the cooling and the heating, 120 and 110 XRD patterns, respectively, were recorded for 10 s each (0.8 s life time and 9.2 s dead time). Calibration of the calorimeter for temperatures was made by melting pure lauric acid (melting temperature = 43.7 °C) as described in Grabielle-Madelmont and Perron (1983).

R software (R Foundation for Statistical Computing, Vienna, Austria) was used to display the XRD patterns as a function of temperature. PeakFit software (Jandel Scientific, Germany) was used to determine the positions and maximum intensities of the Bragg reflections which were fitted with the Gaussian–Lorentzian sum (amplitude) equation (Lopez, Lesieur, Bourgaux, Keller, & Ollivon, 2001).

2.4. Differential scanning calorimetry (DSC)

The thermal properties of control and UFA-enriched TAGs were determined by DSC, using a DSC Q1000 apparatus (TA Instruments, Newcastle, DE). The calibration of the calorimeter was performed with indium standard (melting point = 156.66 °C, ΔH melting = 28.41 J/g). TAG samples were melted, and total sample weights typically ranging from 9 to 16 mg were introduced in 20 μL aluminium pans hermetically sealed after sample introduction. An empty pan was used as a reference. The temperature protocol was the same as for the coupled XRD and DSC experiments. Briefly, the samples were introduced in the calorimeter at 60 °C, kept at this temperature for 5 min to erase all thermal history, then cooled at